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THE COMPARATIVE BIOAVAILABILITY AND *IN VITRO* ASSESSMENT OF SOLID ORAL DOSAGE FORMS OF PARACETAMOL

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by

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ABSTRACT

The dissolution profiles of eight lots of paracetamol tablets representing seven different tablet brands are determined in a USP rotating basket assembly and a stationary basket-rotating paddle apparatus. The *in vitro* data are expressed in terms of dissolution parameters and inter-tablet differences are assessed statistically using analysis of variance (ANOVA) and the Scheffé test. Highly significant differences are observed between a number of the tablets at the 95% confidence level.

Representative tablets from the dissolution rate study and a control dose of paracetamol dissolved in water are subsequently investigated in a 4 x 4 latin square design bioavailability trial. Serum and urine samples are collected and assayed for paracetamol alone (serum) and together with its metabolites (urine) by means of high pressure liquid chromatography. The *in vivo* data are expressed in terms of bioavailability parameters and differences between the test doses are assessed by means of ANOVA. No significant differences are observed between the dosage forms at the 95% confidence level.

INSTRUMENTATION

The High Pressure Liquid Chromatographic System:

The chromatographic system consisted of a model 6000A solvent delivery system (Waters Associates) and a model U6K injector (Waters Associates). The effluent from a 30 cm x 3,9 mm i.d. µBondapak C_{18} reverse phase column (Waters Associates) was monitored with a variable wavelength Pye Unicam LC3 UV detector. The system was equipped with a Hitachi recorder, model QPD_{54}. The temperature of the column was regulated by means of a circulating waterbath (Ultra-Thermostat, model NB-34980, Colora) which pumped water through the jacket of the column. A cooling coil (Tauchkühler, model TK 64, Colora) was immersed in the waterbath to obtain column temperatures that were below room temperature.

Other Instruments Used:

Whirlimixer (Fisons) vortex mixer
Roto-Uni II (BHG) centrifuge
Dri-Block DB-3 and SC-3 Sample Concentrator (Techne) drying block
Ultrasonic (Cole-Parmer) ultrasonic bath
Sartorius Precision 5-figure balance, type 2474

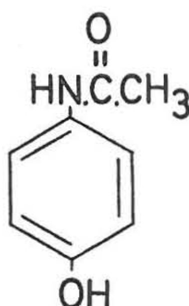
CHAPTER 1

INTRODUCTION

Paracetamol is a commonly used analgesic/antipyretic alternative to aspirin, available under many brand names. It was chosen for this investigation because of its low toxicity at recommended doses, its high therapeutic dose and the fact that methods for the determination of the drug in biological fluids have been reported in the literature.

1.1 PHYSICOCHEMICAL PROPERTIES OF PARACETAMOL

Paracetamol (acetaminophen, N-acetyl-p-aminophenol, 4-hydroxy-acetanilide, p-acetamidophenol or APAP) has the structural formula shown in Figure 1.1.



Mol. mass = 151,16

FIGURE 1.1 Paracetamol

It occurs as white, odourless crystals or a crystalline powder with a bitter taste and a melting point of 169 to 172°C.[1] The solubilities of paracetamol in various solvents are listed in Table 1.1.

Paracetamol is weakly acidic and a saturated aqueous solution has a pH of about 6.[1] Reported pKa values vary from 9,0 to 9,5,[6,7] though a pKa of 10,15 has also been reported.[8]

Pure, dry paracetamol is very stable at temperatures of up to 45°C.[9] If it is exposed to humid conditions, hydrolysis to p-aminophenol occurs. p-Aminophenol readily undergoes further oxidative degradation, which is characterized by a colour change from white, through pink to brown and eventually to black. Degradation in aqueous solutions appears to be acid catalyzed ($t_{\frac{1}{2}}$ is 0,78 years at pH 2) and base catalyzed ($t_{\frac{1}{2}}$ is 2,28 years at pH 9). Solutions at a pH of 5 to 6 are relatively stable ($t_{\frac{1}{2}}$ is 21,8 years at pH 6).[10] Products of the degradation are p-aminophenol and acetic acid.

TABLE 1.1 Reported solubilities of paracetamol

TEMPERATURE	SOLVENT	SOLUBILITY	REFERENCE
20°C	water	1 in 70	1
25°C	water	1 in 86	2
25°C	water	1 in 72	3
37°C	water	1 in 53	4
37°C	water	1 in 50	5
100°C	water	1 in 20	1
20°C	alcohol	1 in 7	1
20°C	methanol	1 in 10	1
20°C	acetone	1 in 13	1
20°C	glycerine	1 in 40	1
20°C	chloroform	1 in 50	1
20°C	propylene glycol	1 in 9	1

Paracetamol absorbs in the ultraviolet (uv) range and has a molar absorptivity of 13,800 in ethanol at 250 nm.[11] It has also been reported to fluoresce in neutral and acidic solutions, but subsequent investigations were unable to confirm this finding and it has been suggested that the fluorescence was due to impurities in the drug or Raman scatter.[12,13]

1.2 PHARMACOKINETICS OF PARACETAMOL

1.2.1 Absorption

Paracetamol is rapidly and completely absorbed following oral administration.[1,14,15] Concomitant administration of food has no significant effect on the amount of drug absorbed, but has a profound effect upon the rate at which the drug is absorbed.[16] Absorption is more rapid in fasting subjects. The above phenomenon would not appear to be due to food-induced changes in metabolism or excretion, as elimination rate constants are similar in the fasting and non-fasting states. It could be due to a lower gastric emptying rate, since paracetamol absorption correlates well with the rate of gastric emptying.[7] Narcotic analgesics, which inhibit gastric emptying, also delay absorption of the drug.[17]

Posture has also been found to influence paracetamol absorption, which is significantly delayed in subjects lying on their left side, as opposed to ambulatory volunteers.[18] This delay in absorption is also thought to be due to a slower gastric emptying rate, as it has been found that the stomach empties more slowly in neonates when they are placed in the supine or left lateral position.

The reason why rapid gastric emptying is associated with rapid paracetamol absorption is uncertain. It has been postulated that rapid gastric emptying could be associated with greater gastrointestinal motility, which in turn could lead to a more rapid disintegration of the ingested tablet. This explanation seems unlikely however, as paracetamol absorption is also related to gastric emptying when the drug is administered in the form of a suspension. More rapid absorption of paracetamol may be due to improved absorption in the small intestines. This presumably results from the larger relative surface area of the latter, since paracetamol is largely unionized in both the stomach and the small intestines and should therefore be equally well absorbed from both sites.[7]

Subjects showing poor absorption characteristics of paracetamol (as judged by a blood level of less than 10 mcg/ml at 45 minutes) have been reported.[19] By the incorporation of sorbitol into the tablets, the number of "poor" absorbers could be reduced from 24 to 6% of the volunteer population. Increasing the dose from 1,0 to 1,5 g was not able to reduce the number of "poor" absorbers to the same extent. The improved absorption did not appear to be due to the formation of an absorbable complex, but could have been due to improved tablet dispersal properties.[20]

Age appears to influence paracetamol absorption. The best absorption occurs in the 30 to 40 year olds, following the ingestion of both crushed and whole tablets. When sorbitol is incorporated in the tablet, the relationship between age and blood level of paracetamol is approximately linear.[19] The study from which these conclusions were drawn reflects rate of absorption rather than extent of absorption however, as the findings are based on a single blood level determination at 45 minutes after the administration of the test dose.

Absorption of paracetamol from suppositories is lower than that from oral dosage forms[21] and has been found to depend upon the dielectric constant

of the suppository base used,[22] as well as the age of certain of the formulations.[23]

1.2.2 Distribution

Gwilt *et al*. studied the distribution of paracetamol after oral administration in man and the dog, which reportedly handle the drug in a similar manner.[24] They demonstrated that paracetamol shows no special preference for any one tissue and, with the exception of fat, is distributed evenly throughout the body. Drug levels in fat are lower than those in the other tissues. This indicates that blood level determinations would give a good assessment of tissue drug levels.

The apparent volume of distribution for unchanged paracetamol has been reported to be 0,78 l/kg[25] and 0,77 l/kg[26] following oral administration. Rawlins *et al*., however, contend that estimates of the volume of distribution following oral administration are unacceptable because the drug is susceptible to "first pass" elimination.[15] They interpreted data obtained after intravenous dosing according to a two compartment open model and found the volume of distribution (central sampling compartment) to be 0,60 $\pm$ 0,07 l/kg and the volume of distribution (peripheral sampling compartment) to be 0,35 $\pm$ 0,02 l/kg. The intercompartmental rate constants were found to be 0,95 $\pm$ 0,27 hr^{-1} and 1,41 $\pm$ 0,18 hr^{-1}.

1.2.3 Plasma Protein Binding

Plasma protein binding of paracetamol is negligible at low concentrations (less than 5%), but increases with increasing concentration.[27] Plasma protein-paracetamol interactions appear to be very weak and do not play a significant role in the bioavailability or elimination of paracetamol at therapeutic levels.[28,29]

1.2.4 Blood Levels

Peak blood levels generally occur 30 to 90 minutes after the ingestion of a single oral dose.[19,30-34] The time at which the peak occurs is subject to various factors such as the presence of food in the gastrointestinal tract and posture. (See Section 1.2.1). Differences in formulation have also been shown to cause a significant shift in the time at which the peak occurs.[32] Maximum paracetamol blood concentrations generally vary from 15 to 40 mcg/ml after a 1,0 g dose.[19,30,32] In man, a significant correlation has been found between blood and saliva levels of the drug.[34,35]

Paracetamol has an elimination half life of 2 to 3 hours.[25,26,30,34,36,44,45] Blood level data are generally interpreted according to a two compartment open model.[15,37,38] A one compartment open model has also been used in cases where the short distributive phase could not be adequately characterized because of a limited number of early samples.[39]

1.2.5 Metabolism

Paracetamol is almost entirely eliminated by biotransformation. The major site of its metabolism is the liver.[39] The main metabolites are paracetamol glucuronide (42 to 76%)[36,40-43] and paracetamol sulphate (19 to 52%),[36,40-43] but trace amounts of other metabolites, including paracetamol cysteine (1%)[42] and paracetamol mercapturic acid (3 to 4%),[41,42] are also formed. Only 3 to 4% of a given dose is excreted as unchanged drug.[36,40,42,43] (See Figure 1.2 for the metabolic pathway of paracetamol.)

Paracetamol glucuronide is formed more rapidly than paracetamol sulphate. Their formation rate constants are 0,15 $\pm$ 0,01 hr^{-1} and 0,08 $\pm$ 0,01 hr^{-1}, respectively.[36] Sulphate conjugation in man appears to be saturated at higher doses.[40,42] The capacity limit varies from subject to subject, but is constant in any one person. In the elderly, conjugation of the drug appears to be impaired.[37]

It has been estimated that 10 to 17 percent of a given dose of paracetamol is metabolized during its first passage through the liver.[28,46] This phenomenon is dose dependant and has been put forward as a reason for the incomplete bioavailability of oral dosage forms which was demonstrated in comparative blood level studies of orally and intravenously administered paracetamol.[15,39] Paracetamol may also be susceptible to a small degree of intestinal metabolism, as it can undergo direct glucuronidation.[30] This could also contribute to the above finding. The total amount of drug (free plus conjugated) excreted in the urine would not be altered by "first pass" elimination or intestinal metabolism.

1.2.5.1 The Role of Metabolism in Paracetamol-induced Hepatic Necrosis

Although paracetamol has a remarkably low toxicity at therapeutic doses, large overdoses cause fatal liver necrosis in man and other animal species.[47,49] Animal studies have demonstrated that hepatic damage is due to a chemically reactive metabolite which is formed by the microsomal

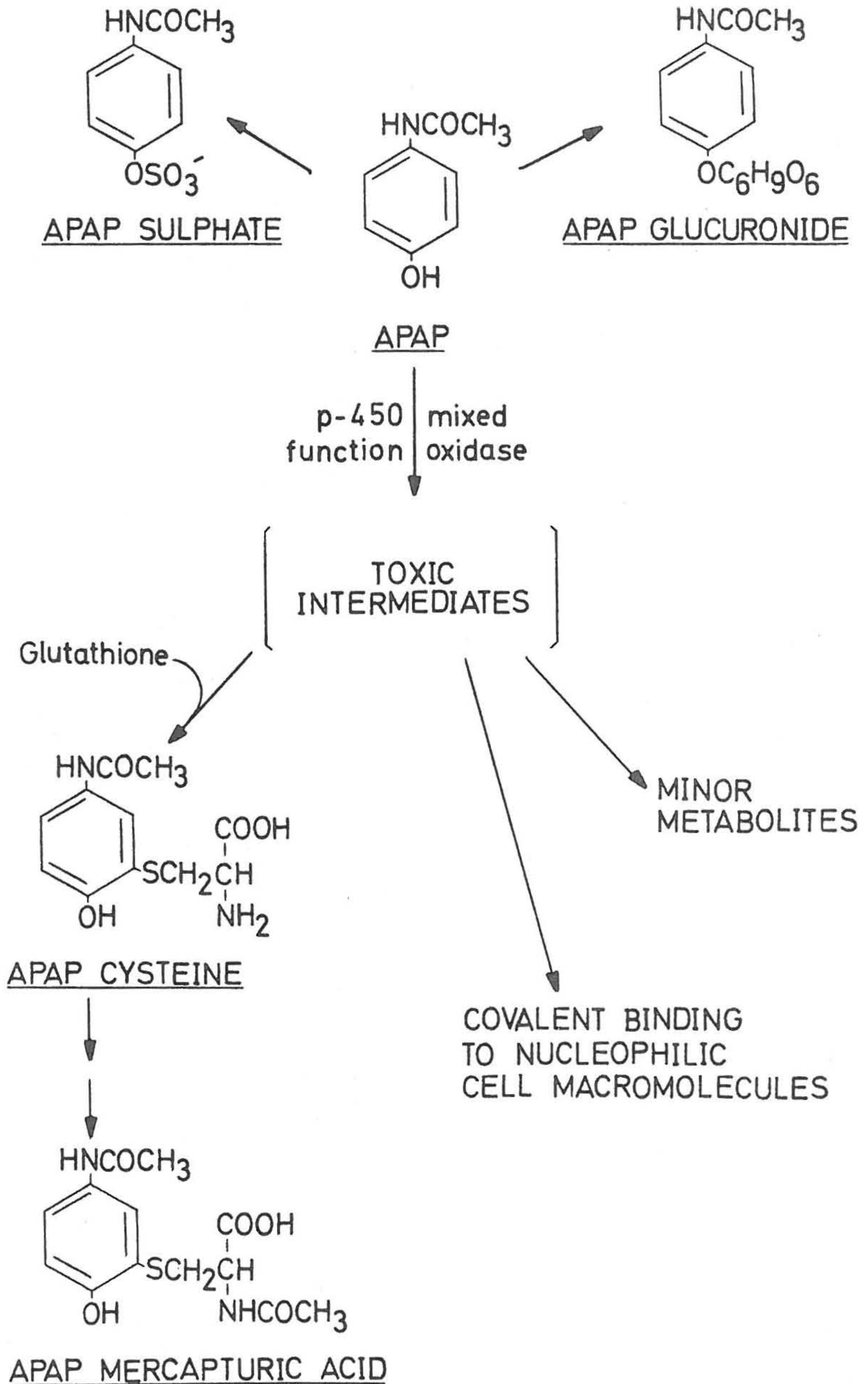


FIGURE 1.2 The metabolic pathway of paracetamol (APAP) in the liver.[41,48]

enzymes, cytochrome p-450 mixed function oxidase.[50-52] The toxic metabolite is an arylating agent and causes damage by binding covalently with hepatocyte macromolecules.[51] Hepatic glutathione is able to protect the liver at therapeutic levels of the drug, since the toxic metabolite appears to bind preferentially to the electrophilic sulphydryl groups of glutathione. After very large doses of paracetamol, the glutathione level of the liver is depleted. When the level has been reduced by more than 70%, the above detoxification mechanism is no longer able to cope and cellular constituents are therefore attacked.[52,53] This leads to cell death. In man, conjugation of drugs with glutathione occurs to a lesser extent than in other animal species,[54] but studies indicate that it does in fact play a protective role similar to the above.[41]

The administration of glutathione precursors, such as cysteine, has been shown to decrease the hepatotoxicity of paracetamol in animal studies.[53] As a result of this finding, the effectiveness of treating paracetamol poisoning in man with compounds that have similar properties to glutathione, has been investigated. The incidence and extent of drug-induced liver damage has been reduced by treating severe paracetamol overdoses with cysteamine,[55,56] methionine,[56,57] acetylcysteine[58,59] and mercaptamine.[60] The protective mechanism has not been elucidated. Antidotes do not appear to act by replacing hepatic glutathione, but may act by inhibiting the formation of the hepatotoxic metabolites.[42] The fact that no new paracetamol metabolites have been detected in treated patients[42] tends to count against the hypothesis that the protective agents act as scavengers combining with the active metabolites.[55]

1.2.5.2 The Effect of Other Drugs on Paracetamol Metabolism

Anti-epileptic drugs, such as phenobarbitone, phenytoin, primidone and carbamazepine, which are known to cause enzyme induction, have been found to increase the elimination rate and total body clearance of paracetamol. This would indicate that the metabolism of paracetamol in man is enhanced by the induction of liver enzymes.[39]

Co-administration of chloramphenicol and paracetamol increases the biological half life of the former from 2 to 3 hours to 18 to 24 hours. This is thought to be due to competitive metabolism.[61]

Salicylamide competitively inhibits the formation of paracetamol sulphate.[40] The capacity-limiting step appears to be the availability of sulphate, as the mutually inhibitory effect is counteracted by the administration of L-cysteine, a source of inorganic sulphate. Results also suggest that the two drugs may competitively inhibit the formation of their respective glucuronide conjugates. No mutual inhibitory effect has been found in the biotransformation of salicylic acid and paracetamol.[45]

1.2.6 Excretion

About 70 to 90% of a given dose of paracetamol can be accounted for in the urine.[7,30,36,40,41,43,62] The renal excretion rate of unchanged paracetamol is slow ($t_{\frac{1}{2}}$ is 59,6 to 62,8 hours).[36,44] The excretion rates of the glucuronide and sulphate conjugates are however rapid ($t_{\frac{1}{2}}$ is 1,22 and 0,6 hours, respectively).[36] Whilst the renal clearance of unchanged paracetamol is increased to a small extent by a faster urine flow, the excretion rates of the conjugates are not affected.[26] Moreover, urinary pH does not affect the excretion of either the unchanged or the conjugated drug.[26,29]

The renal excretion mechanism of paracetamol has been elucidated in the dog. The mechanism is thought to be similar in man. Excretion of unchanged paracetamol involves filtration and reabsorption of the non-ionized form by passive diffusion throughout the nephron. The glucuronide and sulphate conjugates undergo bi-directional tubular transport. In the case of paracetamol sulphate, this occurs in the proximal tubules and appears to be a rate-limited process. The site of glucuronide transport has not yet been identified.[29]

1.3 RECOMMENDED DOSAGE OF PARACETAMOL

The recommended adult dose is 0,5 to 1,0 g every three to four hours. The maximum dose in 24 hours should not exceed 4,0 g.[1]

1.4 ADVERSE EFFECTS

Side effects after therapeutic doses of paracetamol are generally rare and mild. The drug may however cause haematological reactions and skin eruptions.[1]

Toxic doses of paracetamol cause anorexia, nausea, vomiting, abdominal pains and a pale, drawn facies, but no change in the level of consciousness.[63] Complications resulting from paracetamol overdoses include centrilobular hepatic necrosis, renal failure, non-specific cardiac arrhythmia, hypoglycaemia and oesophageal and gastric erosion.[56,58,63]

1.5 CONTRAINDICATIONS

Paracetamol administration is precluded by sensitivity to analgesics derived from aniline. Repeated administration of the drug to patients with anaemia, renal disease or hepatic disease may be contraindicated.[14]

CHAPTER 2

IN VITRO DISSOLUTION RATE DETERMINATIONS

Extensive use is made of solid oral dosage forms in modern medicine. The planning of a drug regimens for a patient is based on the assumption that a stated dose of the medicament will consistently give rise to a certain, predictable blood level of the active ingredient. This is however not always the case and incidents of clinical failure[64,65] and large discrepancies in blood levels[66,67] have been reported, following the replacement of one product with a chemically equivalent product. Occurrences such as these have led to concern in pharmaceutical circles, since existing quality control tests were unable to pick out and exclude the biologically inequivalent products.

Since clinical trials are too expensive for routine quality control, investigators have turned their attention to the development of an in vitro test that will give a reliable indication of the bioavailability of the product. i.e. The rate and extent to which the active ingredients are absorbed and made available at their sites of action.[68] At present, dissolution rate studies are regarded as a potential source of this information. Much work is therefore being done in this field and many methods have been developed for the determination of dissolution rates.

2.1 METHODS AVAILABLE FOR DISSOLUTION RATE DETERMINATIONS

There are numerous methods of determining in vitro dissolution rates in the literature.[69-75] Methods which solely determine the intrinsic dissolution rates of pure drug will not be dealt with here, as disintegration has a profound effect on dissolution characteristics and should therefore be taken into account when one is investigating the dissolution rates of formulated products.[69,74] The study of intrinsic dissolution rates in various dissolution apparatuses is nonetheless a useful means of comparing different systems and assessing the reproducibility of results within a given system.

Dissolution can be studied under conditions of sink and non-sink. Under sink conditions, the solute bulk concentration is very much lower than the equilibrium solubility of the solute at experimental temperatures. Although the solute bulk concentration should be negligible under true

sink conditions, values of up to 10% or even 20% of the equilibrium solubility are accepted in practice.[76] Under non-sink conditions, the solubility limit of the solute is approached more closely and dissolved drug influences the dissolution rate of undissolved drug. This must therefore be taken into account when the results are evaluated. In general, sink conditions would appear to be preferable to non-sink conditions, as the former approximates in vivo conditions more closely than the latter.[72,77]

Dissolution methods can be classified according to a number of criteria, such as the agitational intensity within the system, the achievement of sink or non-sink conditions or the nature of the principle components making up the system. In this case, dissolution methods have been classified according to the way in which convection currents are set up in the dissolution medium.

2.1.1 Agitation of the Entire Dissolution Apparatus

Within this group are systems such as the rotating flask apparatus,[78] the Wruble method,[79,80] the Souder and Ellenbogen method[69,70] and the "shaker" method.[81] (See Figure 2.1 a, b, c, d.) By virtue of the fact that the whole apparatus has to move, size is restricted and these methods are therefore generally used for dissolution studies under conditions of non-sink. The major disadvantage of this class is the difficulty experienced in withdrawing samples without interrupting the dissolution operation.

2.1.2 Movement of the Dosage Form Through the Dissolution Medium

This group consists of modifications of the USP and BP disintegration apparatuses.[82-88] (See Figure 2.1 e.) These methods have the advantage that the apparatus is readily available, information on tablet disintegration is obtained during the dissolution process and sampling can be automated. Results are however sensitive to variations in the oscillation rate. Agitational intensity within the system, which depends upon the volume of dissolution medium used as well as the dimensions of the vessel, also influences results.[69]

2.1.3 Flow of Dissolution Medium Past the Dosage Form

These systems consist of a column in which the dosage form is supported and through which dissolution medium is pumped.[89-93] (See Figure 2.1 f.) The column method is adaptable, sampling can be automated and dosage forms

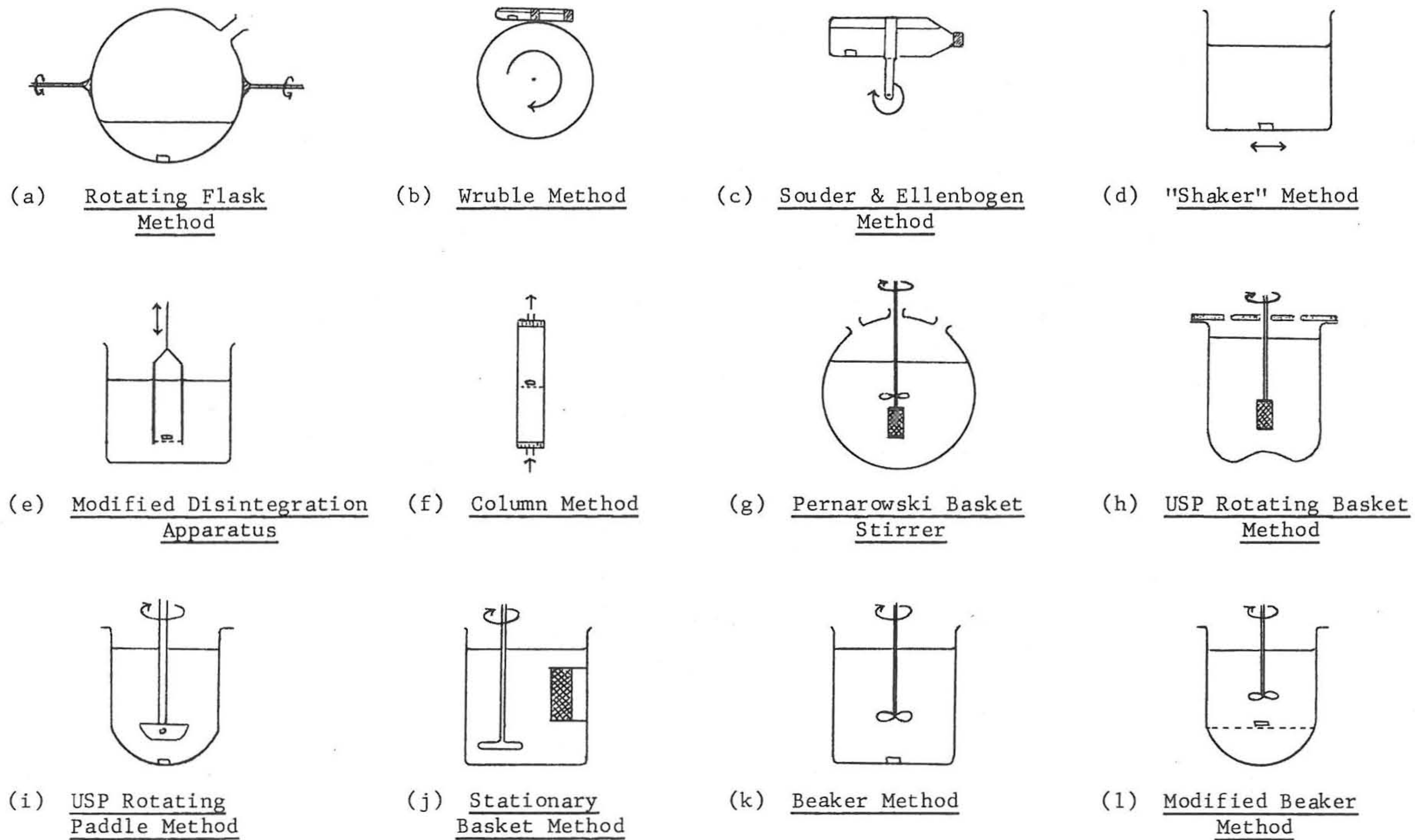


FIGURE 2.1 Methods of determining *in vitro* dissolution rates

are subject to a low intensity of agitation. Moreover, the system can operate under true sink conditions. Variations in the hydrodynamic conditions within the column have however been found to alter the dissolution profiles that are obtained.

2.1.4 Tank Reactor with a Mechanical Agitator

Methods such as the USP rotating basket assembly,[94] the USP rotating paddle method,[94,95] the Pernarowski basket stirrer assembly,[96] the stationary basket method[74,97] and the beaker method[98,99] fall into this group. (See Figure 2.1 g, h, i, j, k, l.) Methods in this class are generally simple to use and can readily be adapted to the testing of a range of drugs. Sampling can be automated, a number of determinations can be run concurrently and sink conditions can be approximated by altering the volume of the dissolution medium used.[100-102]

Since a great number of in vitro dissolution rate studies of solid oral dosage forms have been performed using the beaker method and the USP rotating basket method, they have been singled out for more detailed discussion.

2.1.4.1 The Beaker Method

Levy and Hayes were the first investigators to use this method for assessing the dissolution rate of tablets.[98] The apparatus originally consisted of a 400 ml beaker immersed in a waterbath at $37^{o}C$. The dissolution medium (250 ml) was agitated by means of a 3-bladed polyethylene stirrer, which was accurately centred and immersed to a depth of 27 mm. Since then, many workers have used the original method or a modification of the original method[103-107] and it has enjoyed a relatively high rate of successful in vivo/in vitro correlations.[64,103,104,108-112]

At low stirrer speeds the disintegrated tablet forms a mound at the bottom of the beaker. This phenomenon has been criticized, as it could lead to a large reduction in the surface area available for dissolution.[113] Levy, however, feels that low agitational forces are justified, in spite of mound formation, provided that agitation is sufficient to attain homogeneity for representative sampling.[114] This view is based on studies of the behaviour of tablets in the stomach. By using radio-opaque, barium sulphate tablets he was able to demonstrate[114] that agitational forces in the stomach are so low that the tablet remains as an

aggregate after disintegration and is not distributed uniformly throughout the stomach. In the report, Levy also mentions that other workers had frequently observed the same aggregation effect during gastroscopic investigations following the ingestion of aspirin tablets. Since the location of the mound within the dissolution vessel has been found to influence the dissolution profile of a tablet,[115,124] round-bottomed flasks have replaced the traditional beaker in many cases, to ensure that the mound always forms in the centre of the bottom of the dissolution vessel.[104,108,111]

The marine propeller, used as an agitator in many versions of the beaker method, primarily produces rotational and radial currents at 60 rpm, as does a straight-bladed turbine impeller.[116] The flow pattern set up by the former depends upon the direction in which the propeller is turned. The different flow patterns alter mixing within the dissolution vessel and result in the occurrence of significantly different rates of dissolution.[99] Furthermore, the angle of the propeller blades from the horizontal plane also influences the dissolution rates of solid oral dosage forms. Straight-bladed impellers are not subject to these variations and would therefore appear to be preferable to marine propellers.

Studies on the effect of tablet positioning within the dissolution vessel indicate that this strongly influences the dissolution rate of a drug. Maximum dissolution rates were obtained when the tablet was placed 1 to 2 cm off-centre and lower values were obtained when the tablet was placed at the centre or near the periphery of the vessel.[99] Since it is difficult to keep tablets at a specific place on the vessel bottom unless it is centred by using a round-bottomed flask, the latter practice is recommended in spite of the lower dissolution rates that were observed in this position.

Capsules present a problem in the beaker method as they tend to float on the dissolution medium.[73] Since this tends to alter dissolution characteristics, specialized techniques have to be applied to ensure their complete submersion.[117,118]

2.1.4.2 The Rotating Basket Apparatus

In 1970 a dissolution apparatus based on the Pernarowski basket stirrer assembly was included in the United States Pharmacopoeia. (The apparatus is described in Section 2.2.3.1.) Largely as a result of its official

status, the rotating basket apparatus has been widely used. There are however a number of criticisms levelled against it.

Mattok _et al_. have reported[119] that the steel mesh baskets are corroded by prolonged exposure to simulated gastric fluid. They were able to prevent corrosion by replacing the gastric fluid with a buffered dissolution medium or a less concentrated HCl solution (1 in 100), but found that the new conditions altered dissolution times and led to a different ranking of the tablets tested.

Vibration of the apparatus has been shown to affect dissolution profiles.[120] A six-fold increase in the time required for 50% of the dose to dissolve was experienced when an "extremely smooth" apparatus was used in place of one that was rated as "slightly rough" according to a vibration severity chart.

The lack of rigorous specifications for the official dissolution vessel has been criticized, following the discovery that significantly different results could be obtained when vessels obtained from different sources were used.[121] The vessels all complied with the standards laid down by the USP, but differed from one another by having vessel bottoms with varying degrees of concavity. Discrepancies between vessels were found to be more marked at higher stirrer speeds (150 and 200 rpm) than at lower stirrer speeds (50 and 100 rpm).

Sampling from different points in the dissolution vessel leads to different results.[74,122,123] This appears to be due to a vertical concentration gradient, resulting from inadequate mixing within the system.[123-125] The recommended sampling point is 5 cm from the bottom of the vessel and 1,5 to 2 cm from the side of the basket, since sampling from this position during a study involving a tracer technique, resulted in a profile that rapidly reached a value corresponding to complete distribution of the tracer, without leading to the registration of readings that were greater than this maximum value.[123]

Tablet positioning within the rotating basket has been shown to influence dissolution rates.[99] Tablets placed against the wall of the basket, have a higher dissolution rate than those placed in the centre of the basket. On the vessel bottom, the highest dissolution rate is found in the centre of the vessel. It decreases as the periphery is approached.

Care must be taken when samples are withdrawn by means of a fixed dip tube. Unless liquid is prevented from entering the tube between samples, or the tube is flushed with air before sampling, a stagnant, non-representative column of liquid will make up part of each sample and lead to depressed results.[122] (This does not apply to continuous sampling.) In addition to the above, the sampling tube may alter dissolution results by changing flow patterns within the dissolution vessel. This is especially likely if the tube is large.[123]

The rotating basket has been found to lack reproducibility,[74,126] the wire basket is susceptible to clogging by tablet fragments,[127] difficulty in visualizing the behaviour of tablets during the dissolution process has been experienced[127] and certain dosage forms tend to become entrapped in an air bubble at the top of the basket assembly.[128]

2.2 EXPERIMENTAL

2.2.1 Reagents

Paracetamol (Lot no. 86216) was supplied by Lennon Ltd., S.A. Analytical grade concentrated hydrochloric acid (Lot no. 8533667) was purchased from E. Merck, Darmstadt.
Water was deionized and distilled in glass before use.

2.2.2 Dosage Forms

Eight lots of tablets, representing seven different brands, were used in the study. Details of the tablets are given in Table 2.1.

TABLE 2.1 Formulations used in the dissolution rate study.

TRADE NAME	BATCH No.	MANUFACTURER	PARACETAMOL CONTENT (% label claim)
Dolorol	7654 K	NHP	98,84 ± 0,19
Napamol	609182	Alex Lipworth	96,34 ± 0,24
Norstan-Paracetamol	805	Noristan	97,53 ± 0,14
Panado	X994 PK	Winthrop	97,86 ± 1,24
Paracetamol	32449	Petersen	97,95 ± 0,12
Paracetamol	32978	Petersen	98,82 ± 0,23
Repamol	TF 704	Rep-pharm	97,58 ± 0,15
Paracetamol	762015	Propan	98,78 ± 0,06

All of the dosage forms were white, uncoated, compressed tablets with a label strength of 500 mg. With the exception of one lot (Norstan-Paracetamol), the tablets were obtained from hospital or retail outlets. They were assayed for tablet content of active ingredient[129] and were found to comply with the BP specifications.

2.2.3 Dissolution Methodology

2.2.3.1 The Rotating Basket Apparatus

The rotating basket assembly is an official United States Pharmacopoeia apparatus.[94] It consists of a 1000 ml dissolution vessel with a slightly concave bottom. A cylindrical, 40 mesh, stainless steel basket is attached to a stainless steel shaft and rotated in the dissolution medium. The dissolution vessel is covered with a perspex lid containing a central opening for the basket assembly and three peripheral openings for sampling and fluid replacement. (See Figure 2.2.)

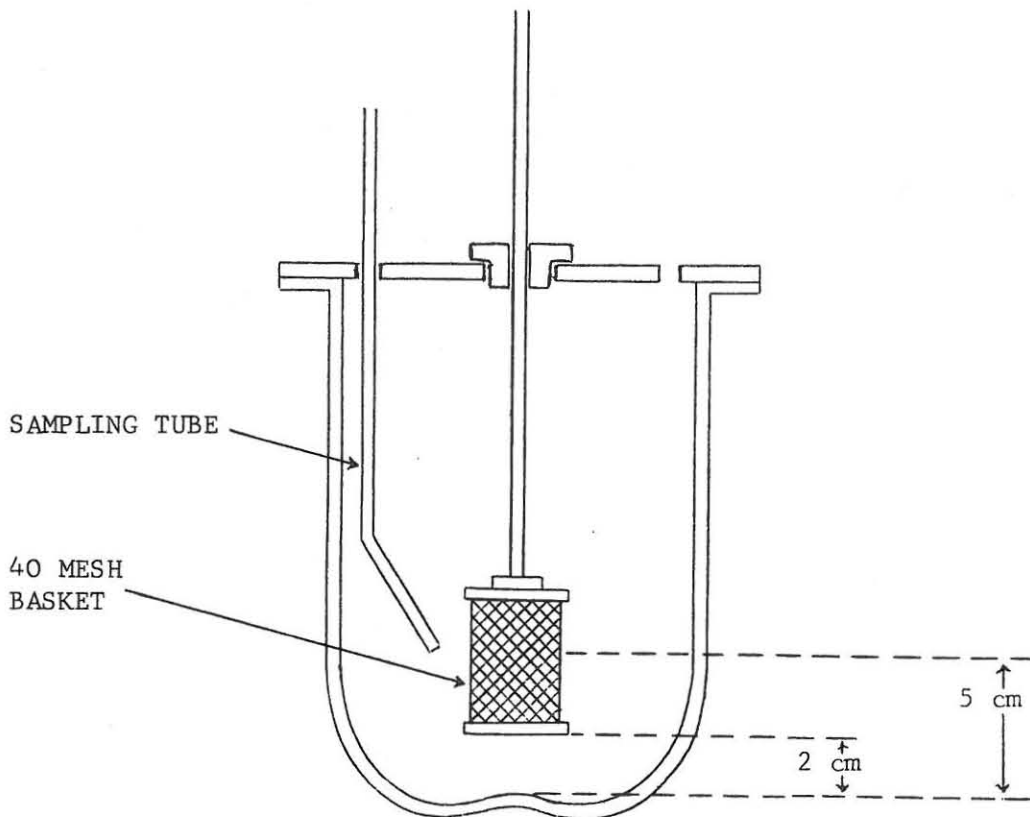


FIGURE 2.2 The USP rotating basket apparatus

A multiple spindle drive apparatus (Model QC 72R-230 B, Hansen Research Corp., Northridge, California) was used. The original waterbath was replaced by a clear, perspex bath, which was heated by a Thermomix-1480 circulating pump (B.Braun Melsungen AG.). The pump was mounted in such a way that vibrations were not transmitted to the rest of the apparatus.

The dissolution medium consisted of 1000 ml of 0,1N HCl, the stirrer speed was set at 100 rpm and the system was maintained at a temperature of 37°C. For each determination a single tablet was placed in the wire basket. At zero time the basket was immersed in the dissolution fluid to a position 2 cm from the vessel bottom and the clutch was released to initiate stirring.

In one study, samples were withdrawn and analysed manually and in another, sampling and analysis were automated. The two procedures are described separately below.

(i) Manual Sampling and Analysis

A clean 10 ml pipette was used to withdraw each sample and care was taken to ensure that all of the samples were removed from the same point in the dissolution vessel, 5 cm from the bottom of the vessel and 2 cm from the basket. Fluid was replaced by means of an automatic dispenser, calibrated to deliver 10 ml of 0,1N HCl. Samples were placed in clean, dry, screw-cap bottles and stored in a cool, dark cupboard until they could be analysed. Storage time did not exceed 3 hours.

Analysis was performed spectrophotometrically using a Beckman Acta MVI uv-visible spectrophotometer. A 2,0 ml aliquot of each sample was diluted to 100,0 ml with 0,1N HCl and the absorbance of the resultant solution was read at 241,5 nm (slit width 3 mm; reference energy 400). The absorbance readings were converted to the cumulative fraction of the drug dissolved, 'm', at each sampling time, by means of a computer program based on equation 2.1.

$$m = \left(\left(\frac{A_n}{a} \times 50 \right) + \sum_{n=1}^{n-1} \frac{A_{(n-1)}}{2a} \right) \Big/ 0,5 \qquad (2.1)$$

where 'A_n' is the absorbance reading of the nth sample, '$A_{(n-1)}$' is the absorbance reading of the (n-1)th sample and 'a' is the absorptivity

of paracetamol. (a = 64,481; r = 0,99999.) The program corrected for dilution factors as well as the cumulative amount of drug removed in previous samples.

Six tablets from each tablet lot were tested and the order of testing was randomized to eliminate sequential effects. More than one tablet could be tested at a time by staggering the starting times. Figure 2.5 shows the dissolution profiles that were obtained and the results are listed in Table 2.2.

(ii) Automated Sampling and Analysis

Automation of the dissolution process was achieved by incorporating a Technicon Autoanalyzer II Proportioning Pump III, fitted with SMA flow rated tubes into the system.

Sampling was continuous at a rate of 0,16 ml/min. The sampling tube was kept in position within the dissolution vessel by means of a glass support rod, which was clamped in a retort stand. Solid particles were removed from the sample by a cottonwool plug in the mouth of the sampling tube. The plug was renewed for each determination. Prior to being mixed in a mixing coil, the sample was diluted with 0,1N HCl (6,0 ml/min.) and segmented within the transmission tubing by air bubbles. The air bubbles were introduced into the stream every 2 seconds via a flow rated pump tube (0,32 ml/min.). The diluted sample was pumped through the sample cell of the spectrophotometer at a rate of 2,0 ml/min. Bubbles were removed from the sample stream just before it entered the flow cell. 0,1N HCl was pumped through the reference cell at a rate of 2,0 ml/min. Effluent from the flow cells and debubbling units was run to waste. A flow diagram of the system is presented in Figure 2.3.

The absorbance of the diluted sample was read at 241,5 nm (slit width 3 mm; reference energy 400) and a trace of absorbance *versus* time was generated. Since the relationship between absorbance and concentration was shown to be linear over the range of interest, the fraction of the dose dissolved at each point was calculated directly from the dissolution trace. Complete dissolution was judged to have occurred when undissolved particles could no longer be seen in the dissolution vessel and when the dissolution trace had levelled off. The fraction dissolved at a given time was calculated by expressing the height of the dissolution trace at that point as a fraction of its maximum or "plateau" height.

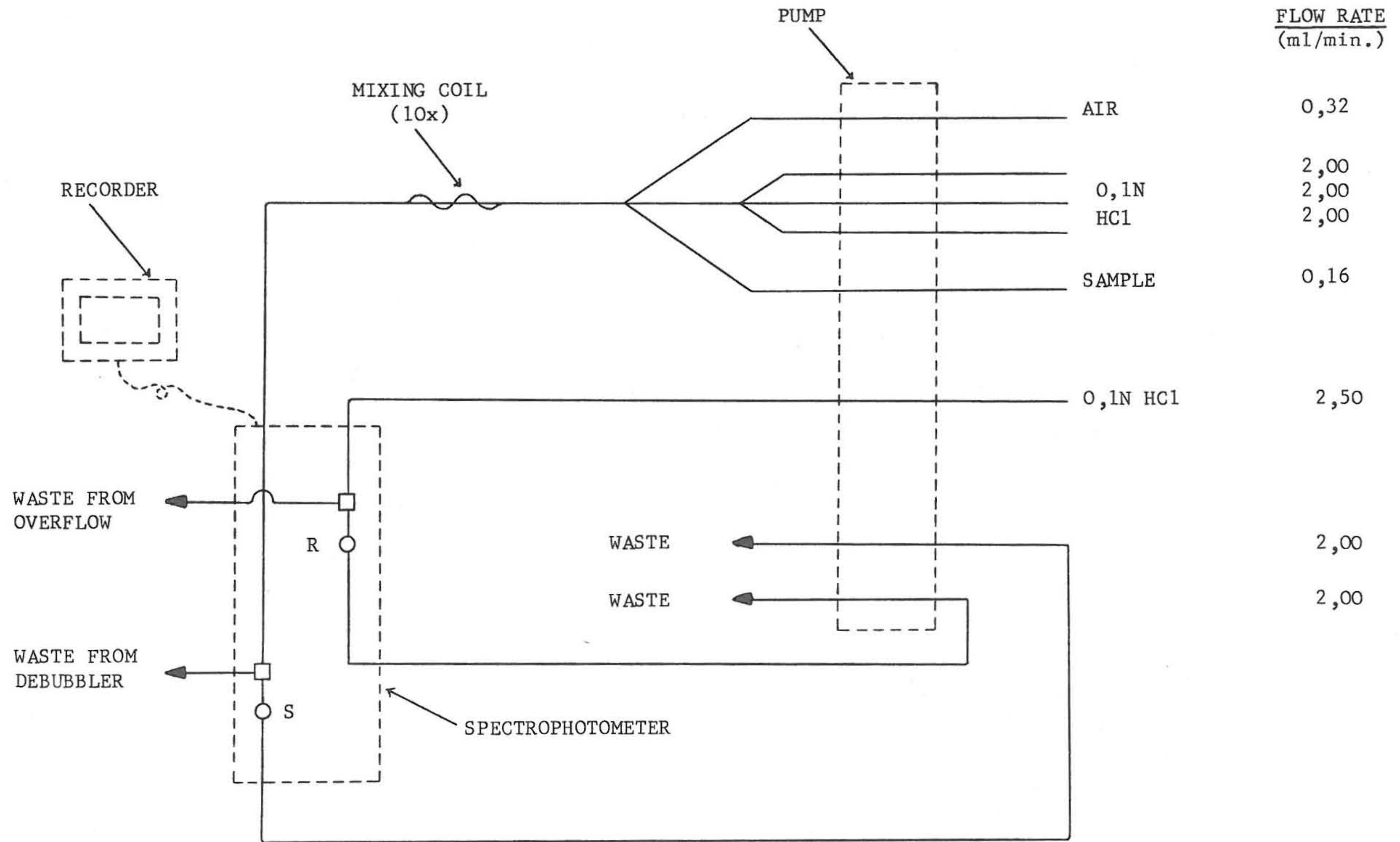


FIGURE 2.3 Flow diagram of the automated dissolution apparatus

There was a lag period before a response was registered by the spectrophotometer. This delay represented the time it took for a given sample to travel from the dissolution vessel to the flow cell, and zero time on the dissolution trace therefore had to be adjusted accordingly. The lag time was checked at regular intervals and was found to be constant for the duration of the determinations.

Four tablets from each tablet lot were tested in a randomized order to avoid sequential effects. Results are listed in Table 2.3 and Figure 2.6 shows the dissolution profiles that were obtained.

2.2.3.2 The Stationary Basket-Rotating Paddle Apparatus

This is the method of Ganderton *et al.*,[130] as modified by Goosens.[131] It consists of a 2 litre beaker with a perspex lid. The stirrer shaft of a perspex paddle passes through the centre of the lid and peripheral openings are provided for sampling, fluid replacement and the housing of a perspex dosage holder. (See Figure 2.4.)

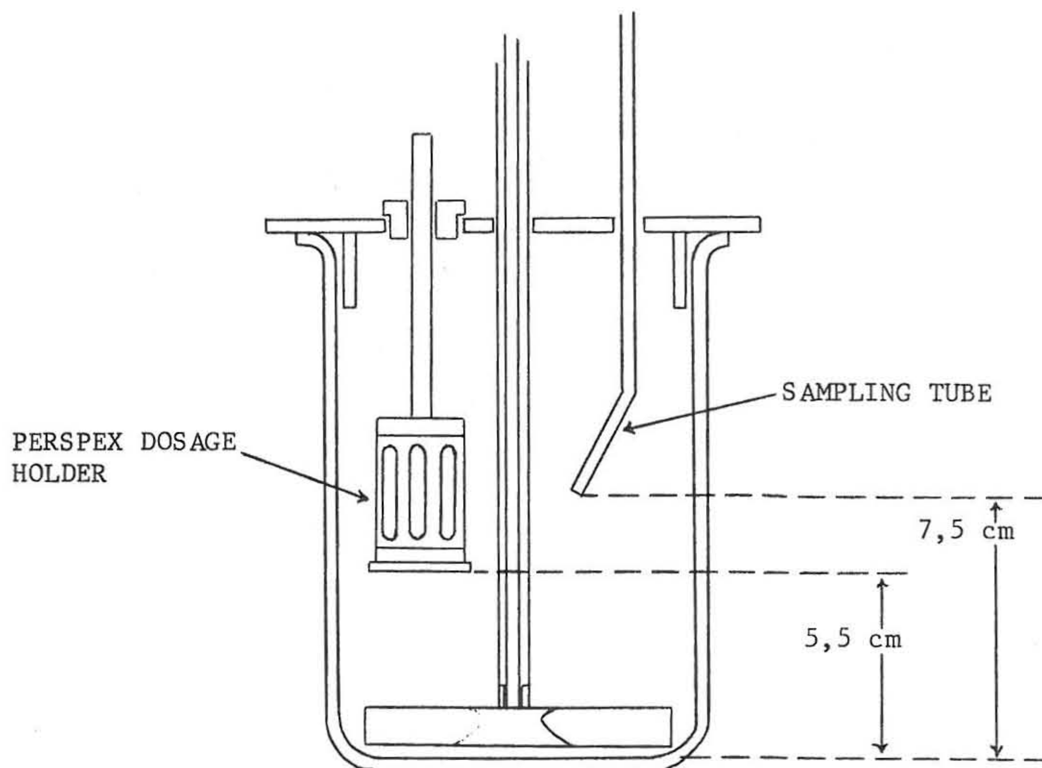


FIGURE 2.4 The stationary basket-rotating paddle apparatus

The multiple spindle drive apparatus used in the rotating basket method (Section 2.2.3.1), was modified in such a way that it could accommodate the 2 litre beakers and drive the paddles.

The dissolution medium consisted of 1500 ml of 0,1N HCl, the stirrer speed was set at 100 rpm and the system was maintained at a temperature of 37°C. For each determination a single tablet was placed in the perspex dosage holder. The clutch was released to initiate stirring and the tablet was immersed in the dissolution medium at zero time. Sampling and analysis was automated as in Section 2.2.3.1 (ii) and the fraction of the dose dissolved at a given time was calculated directly from the absorbance versus time trace. Four tablets from each tablet lot were tested in a randomized order. Results are listed in Table 2.4 and Figure 2.7 shows the dissolution profiles that were obtained.

2.3 RESULTS AND DISCUSSION

The dissolution curves obtained from the manual and automated USP rotating basket assemblies were almost identical (Figures 2.5 and 2.6). This would tend to confirm that not replacing dissolution medium in the latter method had no significant effect on results. During the automated dissolution process, the disintegration pattern of the tablets was noted and was found to have a definite effect on the dissolution profiles of the dosage forms. The tablets representing the four, closely grouped dissolution curves on the extreme left (Figure 2.6), all disintegrated completely within two to four minutes and yielded steep curves. The Propan paracetamol tablets initially disintegrated slowly, but after about four minutes, the remaining tablet core disintegrated so rapidly that it appeared to melt away. This behaviour explains the S-shaped curve that was obtained. Napamol disintegrated rapidly and on these grounds, one would have expected the curve to appear amongst the closely grouped, steep curves on the extreme left. The granules however remained intact for a long time after disintegration and this resulted in a curve with a relatively gentle slope. Panado disintegrated relatively rapidly for a short while, after which it slowed down to a steady rate. This is reflected by the dissolution profile, which has a relatively steep initial slope and then acquires a more gradual slope. Repamol did not disintegrate to a significant

extent. Dissolution therefore occurred largely from the tablet surface and this resulted in a profile with a relatively gentle slope. Dissolution in the stationary basket-rotating paddle apparatus was faster, overall, than that in the rotating basket method. (Figure 2.7). This could be due to greater agitational forces within the former apparatus. With the exception of Panado and Napamol, the relative positions of the dissolution profiles were largely unchanged, as were the disintegration patterns of the tablets. In the stationary basket apparatus, Panado disintegrated rapidly and the curve appeared amongst the closely grouped curves on the extreme left. The disintegration pattern of Napamol was unaltered. The steeper curve of Napamol would therefore appear to be due to enhanced dissolution of the granules in the system.

Automation of the dissolution system had distinct advantages over the manual sampling method. It yielded a continuous, well-characterized dissolution profile, from which the cumulative fraction of drug dissolved could be calculated at any chosen time interval. In this study, the fraction dissolved was initially calculated every twelve seconds. As the cumulative fraction dissolved approached unity and the variation between consecutive readings became less than 0,005, the time interval between readings was increased to one and then to five minutes. From manual sampling methods, a finite number of data points are obtained. In cases where the available information proves inadequate for subsequent data processing, the acquisition of additional data points generally necessitates a repetition of the determination. Automation also enabled three to four times as many dissolution studies to be performed in a day, since the entire determination took only as long as the dissolution process of the tablet. In the manual method, additional time was needed for the analysis of samples.

In order to obtain good dissolution traces, it was found to be important to have segmentation within the transmission tubing by the introduction of an air bubble at regular time intervals. The bubbles were required to divide the fluid stream into discrete samples, which are then unable to merge with one another on the way to the flow cell because of the scrubbing action of the bubbles on the tube walls. Furthermore, segmentation was required for the achievement of adequate mixing in the mixing coil. The importance of regular air segmentation is illustrated by Figure 2.8, which shows a dissolution trace obtained

TABLE 2.2 Results from the manual USP rotating basket apparatus

Each result represents the average of six determinations. The standard deviation appears below each mean in parentheses. Time intervals are expressed in minutes.

TABLET	FRACTION DISSOLVED AT EACH TIME INTERVAL											
	2,5	5,0	7,5	10,0	12,5	15,0	20,0	25,0	30,0	40,0	50,0	60,0
Dolorol	0,617 (0,221)	0,907 (0,031)	0,961 (0,017)	0,982 (0,006)	-	0,997 (0,002)	0,999 (0,002)	1,000 (0,000)	1,000 (0,000)	1,000 (0,000)	-	1,000 (0,000)
Napamol	-	0,360 (0,126)	-	0,656 (0,189)	-	0,824 (0,188)	0,905 (0,144)	0,953 (0,080)	0,973 (0,050)	0,992 (0,020)	0,999 (0,002)	1,000 (0,000)
Norstan-Paracetamol	0,578 (0,080)	0,853 (0,060)	0,935 (0,027)	0,970 (0,012)	-	0,989 (0,007)	0,996 (0,004)	0,999 (0,001)	1,000 (0,001)	1,000 (0,000)	-	1,000 (0,000)
Panado	-	0,358 (0,067)	-	0,624 (0,139)	-	0,800 (0,138)	0,893 (0,099)	0,948 (0,058)	0,976 (0,032)	0,993 (0,013)	1,000 (0,001)	1,000 (0,000)
Paracetamol (Petersen B/N 32449)	0,814 (0,059)	0,946 (0,015)	0,979 (0,005)	0,989 (0,004)	-	0,997 (0,004)	0,999 (0,001)	1,000 (0,000)	1,000 (0,000)	1,000 (0,000)	-	1,000 (0,000)
Paracetamol (Petersen B/N 32978)	0,712 (0,188)	0,900 (0,059)	0,960 (0,028)	0,985 (0,011)	-	0,998 (0,002)	1,000 (0,001)	1,000 (0,000)	1,000 (0,000)	1,000 (0,000)	-	1,000 (0,000)
Paracetamol (Propan)	0,176 (0,016)	0,379 (0,089)	0,733 (0,106)	0,855 (0,075)	0,908 (0,051)	0,940 (0,033)	0,974 (0,015)	0,987 (0,008)	0,995 (0,006)	0,999 (0,002)	1,000 (0,000)	1,000 (0,000)
Repamol	-	0,278 (0,063)	-	0,453 (0,099)	-	0,590 (0,103)	0,714 (0,097)	0,827 (0,098)	0,907 (0,070)	0,973 (0,044)	-	1,000 (0,000)

TABLE 2.3 Results from the automated USP rotating basket apparatus

Each result represents the average of four determinations. The standard deviation appears below each mean in parentheses. Time intervals are expressed in minutes.

	FRACTION DISSOLVED AT EACH TIME INTERVAL										
	0,2	0,4	0,6	0,8	1,0	1,2	1,4	1,6	1,8	2,0	2,2
Dolorol	0,008	0,030	0,064	0,099	0,139	0,186	0,237	0,297	0,362	0,431	0,508
	(0,001)	(0,003)	(0,011)	(0,023)	(0,039)	(0,053)	(0,063)	(0,075)	(0,091)	(0,101)	(0,097)
Napamol	0,015	0,043	0,071	0,098	0,117	0,135	0,149	0,164	0,177	0,191	0,204
	(0,009)	(0,013)	(0,019)	(0,022)	(0,027)	(0,031)	(0,034)	(0,036)	(0,038)	(0,038)	(0,041)
Norstan-	0,010	0,042	0,097	0,153	0,206	0,255	0,299	0,339	0,377	0,421	0,461
Paracetamol	(0,007)	(0,012)	(0,014)	(0,011)	(0,015)	(0,025)	(0,027)	(0,033)	(0,049)	(0,046)	(0,048)
Panado	0,004	0,022	0,051	0,081	0,109	0,135	0,161	0,185	0,205	0,226	0,244
	(0,001)	(0,008)	(0,025)	(0,038)	(0,048)	(0,058)	(0,072)	(0,086)	(0,093)	(0,104)	(0,113)
Paracetamol	0,015	0,062	0,114	0,168	0,232	0,320	0,424	0,530	0,606	0,663	0,715
(Petersen B/N 32449)	(0,009)	(0,022)	(0,028)	(0,034)	(0,047)	(0,072)	(0,081)	(0,077)	(0,075)	(0,072)	(0,065)
Paracetamol	0,013	0,045	0,089	0,139	0,191	0,251	0,335	0,430	0,518	0,595	0,659
(Petersen B/N 32978)	(0,008)	(0,015)	(0,025)	(0,036)	(0,050)	(0,064)	(0,086)	(0,106)	(0,110)	(0,108)	(0,095)
Paracetamol	0,004	0,015	0,029	0,043	0,055	0,066	0,078	0,091	0,102	0,115	0,127
(Propan)	(0,004)	(0,009)	(0,012)	(0,014)	(0,016)	(0,018)	(0,019)	(0,021)	(0,024)	(0,025)	(0,028)
Repamol	0,013	0,033	0,054	0,073	0,091	0,107	0,121	0,134	0,146	0,157	0,168
	(0,011)	(0,012)	(0,011)	(0,017)	(0,026)	(0,032)	(0,035)	(0,039)	(0,040)	(0,042)	(0,043)

TABLE 2.3 Continued

TABLET	FRACTION DISSOLVED AT EACH TIME INTERVAL										
	2,4	2,6	2,8	3,0	3,2	3,4	3,6	3,8	4,0	4,2	4,4
Dolorol	0,568 (0,095)	0,616 (0,100)	0,655 (0,092)	0,695 (0,080)	0,731 (0,069)	0,760 (0,061)	0,783 (0,057)	0,803 (0,052)	0,819 (0,048)	0,833 (0,045)	0,847 (0,042)
Napamol	0,217 (0,041)	0,229 (0,040)	0,242 (0,041)	0,255 (0,040)	0,265 (0,040)	0,276 (0,040)	0,287 (0,039)	0,298 (0,040)	0,309 (0,043)	0,321 (0,047)	0,333 (0,052)
Norstan-Paracetamol	0,500 (0,045)	0,536 (0,041)	0,568 (0,039)	0,601 (0,035)	0,629 (0,037)	0,656 (0,040)	0,680 (0,042)	0,702 (0,040)	0,725 (0,038)	0,748 (0,035)	0,768 (0,032)
Panado	0,261 (0,123)	0,278 (0,132)	0,292 (0,138)	0,307 (0,145)	-	-	-	-	0,369 (0,168)	-	-
Paracetamol (Petersen B/N 32449)	0,759 (0,056)	0,793 (0,046)	0,820 (0,041)	0,841 (0,039)	0,858 (0,037)	0,873 (0,033)	0,886 (0,030)	0,898 (0,028)	0,909 (0,026)	0,918 (0,023)	0,927 (0,021)
Paracetamol (Petersen B/N 32978)	0,708 (0,087)	0,746 (0,082)	0,776 (0,075)	0,804 (0,066)	0,824 (0,061)	0,844 (0,055)	0,859 (0,051)	0,872 (0,047)	0,884 (0,044)	0,893 (0,041)	0,903 (0,037)
Paracetamol (Propan)	0,137 (0,030)	0,147 (0,033)	0,158 (0,035)	0,171 (0,037)	0,184 (0,040)	0,197 (0,042)	0,210 (0,043)	0,222 (0,046)	0,234 (0,049)	0,248 (0,053)	0,264 (0,056)
Repamol	0,179 (0,045)	0,189 (0,047)	0,199 (0,049)	0,208 (0,052)	-	-	-	-	0,256 (0,063)	-	-

TABLE 2.3 Continued

TABLET	FRACTION DISSOLVED AT EACH TIME INTERVAL										
	4,6	4,8	5,0	5,2	5,4	5,6	5,8	6,0	6,2	6,4	6,6
Dolorol	0,858 (0,038)	0,868 (0,035)	0,878 (0,033)	-	-	-	-	0,915 (0,024)	-	-	-
Napamol	0,345 (0,058)	0,355 (0,061)	0,366 (0,063)	0,377 (0,067)	0,387 (0,071)	0,398 (0,076)	0,410 (0,083)	0,422 (0,089)	-	-	-
Norstan-Paracetamol	0,785 (0,030)	0,800 (0,029)	0,813 (0,028)	0,825 (0,027)	0,837 (0,026)	0,847 (0,024)	0,856 (0,023)	0,866 (0,023)	0,875 (0,023)	0,883 (0,023)	0,890 (0,022)
Panado	-	-	0,422 (0,183)	-	-	-	-	0,467 (0,194)	-	-	-
Paracetamol (Petersen B/N 32449)	0,934 (0,019)	0,941 (0,016)	0,947 (0,014)	-	-	-	-	0,964 (0,008)	-	-	-
Paracetamol (Petersen B/N 32978)	0,910 (0,036)	0,918 (0,032)	0,924 (0,029)	0,930 (0,027)	0,936 (0,024)	0,941 (0,021)	0,945 (0,019)	0,949 (0,018)	-	-	-
Paracetamol (Propan)	0,279 (0,063)	0,299 (0,070)	0,317 (0,077)	0,335 (0,081)	0,354 (0,085)	0,376 (0,085)	0,399 (0,084)	0,426 (0,079)	0,457 (0,073)	0,494 (0,061)	0,532 (0,048)
Repamol	-	-	0,301 (0,076)	-	-	-	-	0,347 (0,089)	-	-	-

TABLE 2.3 Continued

TABLET	FRACTION DISSOLVED AT EACH TIME INTERVAL										
	6,8	7,0	8,0	9,0	10,0	11,0	12,0	13,0	14,0	15,0	16,0
Dolorol	-	0,940 (0,017)	0,958 (0,012)	0,969 (0,008)	0,977 (0,006)	0,982 (0,004)	0,987 (0,003)	0,991 (0,002)	0,993 (0,002)	0,995 (0,001)	0,996 (0,002)
Napamol	-	0,473 (0,111)	0,521 (0,122)	0,571 (0,126)	0,620 (0,131)	0,664 (0,133)	0,701 (0,137)	0,737 (0,141)	0,766 (0,142)	0,795 (0,145)	0,818 (0,145)
Norstan-Paracetamol	0,897 (0,021)	0,902 (0,020)	0,928 (0,016)	0,945 (0,011)	0,958 (0,010)	0,967 (0,008)	0,974 (0,008)	0,979 (0,006)	0,984 (0,005)	0,987 (0,005)	0,989 (0,004)
Panado	-	0,507 (0,199)	0,545 (0,201)	0,581 (0,199)	0,613 (0,199)	0,643 (0,199)	0,669 (0,197)	0,694 (0,194)	0,718 (0,187)	0,739 (0,179)	0,761 (0,170)
Paracetamol (Petersen B/N 32449)	-	0,976 (0,006)	0,984 (0,005)	0,991 (0,004)	0,994 (0,003)	0,997 (0,003)	0,999 (0,002)	1,000 (0,001)	-	-	-
Paracetamol (Petersen B/N 32978)	-	0,964 (0,012)	0,976 (0,008)	0,984 (0,005)	0,989 (0,004)	0,993 (0,003)	0,995 (0,002)	0,997 (0,002)	0,999 (0,001)	0,999 (0,001)	1,000 (0,000)
Paracetamol (Propan)	0,565 (0,034)	0,599 (0,025)	0,711 (0,041)	0,772 (0,038)	0,820 (0,038)	0,856 (0,038)	0,884 (0,038)	0,906 (0,035)	0,923 (0,032)	0,942 (0,017)	0,953 (0,012)
Repamol	-	0,392 (0,106)	0,434 (0,115)	0,472 (0,118)	0,504 (0,118)	0,536 (0,120)	0,568 (0,122)	0,600 (0,122)	0,632 (0,122)	0,662 (0,122)	0,690 (0,121)

TABLE 2.3 Continued

TABLET	FRACTION DISSOLVED AT EACH TIME INTERVAL										
	17,0	18,0	19,0	20,0	21,0	22,0	23,0	24,0	25,0	26,0	27,0
Dolorol	0,998 (0,001)	0,999 (0,001)	1,000 (0,001)	-	-	-	-	-	-	-	-
Napamol	0,839 (0,143)	0,855 (0,142)	0,867 (0,139)	0,880 (0,134)	0,891 (0,127)	0,902 (0,121)	0,911 (0,116)	0,919 (0,110)	0,925 (0,105)	-	-
Norstan-Paracetamol	0,992 (0,004)	0,993 (0,003)	0,995 (0,002)	0,997 (0,003)	-	-	-	-	1,000 (0,000)	-	-
Panado	0,781 (0,159)	0,802 (0,147)	0,820 (0,134)	0,839 (0,120)	0,862 (0,108)	0,885 (0,102)	0,902 (0,095)	0,913 (0,089)	0,923 (0,083)	0,930 (0,078)	0,936 (0,074)
Paracetamol (Petersen B/N 32449)	-	-	-	-	-	-	-	-	-	-	-
Paracetamol (Petersen B/N 32978)	-	-	-	-	-	-	-	-	-	-	-
Paracetamol (Propan)	0,962 (0,010)	0,969 (0,007)	0,976 (0,006)	0,980 (0,005)	-	-	-	-	0,993 (0,003)	-	-
Repamol	0,718 (0,121)	0,746 (0,122)	0,772 (0,122)	0,799 (0,125)	0,824 (0,124)	0,844 (0,122)	0,864 (0,118)	0,881 (0,114)	0,895 (0,109)	0,911 (0,101)	0,925 (0,091)

TABLE 2.3 Continued

TABLET	FRACTION DISSOLVED AT EACH TIME INTERVAL						
	28,0	29,0	30,0	35,0	40,0	45,0	50,0
Dolorol	-	-	-	-	-	-	-
Napamol	-	-	0,954 (0,075)	0,980 (0,036)	0,992 (0,016)	0,997 (0,006)	1,000 (0,001)
Norstan-Paracetamol	-	-	-	-	-	-	-
Panado	0,941 (0,070)	0,946 (0,066)	0,951 (0,062)	0,990 (0,012)	0,999 (0,002)	1,000 (0,000)	-
Paracetamol (Petersen B/N 32449)	-	-	-	-	-	-	-
Paracetamol (Petersen B/N 32978)	-	-	-	-	-	-	-
Paracetamol (Propan)	-	-	0,999 (0,002)	1,000 (0,000)	-	-	-
Repamol	0,937 (0,081)	0,948 (0,071)	0,959 (0,061)	0,990 (0,020)	0,998 (0,004)	1,000 (0,000)	-

TABLE 2.4 Results from the automated stationary basket-rotating paddle apparatus

Each result represents the average of four determinations. The standard deviation appears below each mean in parentheses. Time intervals are expressed in minutes.

TABLET	FRACTION DISSOLVED AT EACH TIME INTERVAL											
	0,2	0,4	0,6	0,8	1,0	1,2	1,4	1,6	1,8	2,0	2,2	2,4
Dolorol	0,027	0,100	0,203	0,316	0,439	0,552	0,657	0,745	0,806	0,850	0,879	0,902
	(0,012)	(0,034)	(0,053)	(0,066)	(0,076)	(0,074)	(0,072)	(0,058)	(0,047)	(0,036)	(0,027)	(0,019)
Napamol	0,028	0,099	0,206	0,334	0,450	0,536	0,596	0,642	0,681	0,714	0,739	0,761
	(0,012)	(0,021)	(0,031)	(0,041)	(0,044)	(0,038)	(0,031)	(0,029)	(0,025)	(0,019)	(0,018)	(0,016)
Norstan-	0,013	0,058	0,139	0,258	0,369	0,451	0,514	0,567	0,613	0,661	0,702	0,742
Paracetamol	(0,013)	(0,037)	(0,065)	(0,075)	(0,089)	(0,075)	(0,062)	(0,051)	(0,044)	(0,036)	(0,027)	(0,024)
Panado	0,031	0,109	0,231	0,376	0,512	0,623	0,711	0,770	0,818	0,856	0,882	0,904
	(0,027)	(0,067)	(0,108)	(0,139)	(0,156)	(0,147)	(0,128)	(0,111)	(0,091)	(0,073)	(0,057)	(0,045)
Paracetamol	0,042	0,116	0,217	0,347	0,492	0,629	0,719	0,779	0,820	0,852	0,875	0,894
(Petersen B/N 32449)	(0,017)	(0,032)	(0,051)	(0,075)	(0,086)	(0,076)	(0,066)	(0,057)	(0,046)	(0,038)	(0,031)	(0,027)
Paracetamol	0,029	0,100	0,205	0,316	0,432	0,550	0,644	0,720	0,778	0,822	0,849	0,872
(Petersen B/N 32978)	(0,020)	(0,038)	(0,047)	(0,052)	(0,064)	(0,070)	(0,074)	(0,061)	(0,042)	(0,029)	(0,022)	(0,016)
Paracetamol	0,014	0,040	0,075	0,112	0,149	0,191	0,231	0,268	0,305	0,345	0,384	0,422
(Propan)	(0,009)	(0,016)	(0,022)	(0,023)	(0,022)	(0,026)	(0,031)	(0,032)	(0,032)	(0,030)	(0,031)	(0,033)
Repamol	0,007	0,027	0,056	0,082	0,104	0,126	0,146	0,164	0,183	0,202	0,218	0,233
	(0,002)	(0,005)	(0,006)	(0,005)	(0,005)	(0,007)	(0,008)	(0,009)	(0,011)	(0,011)	(0,014)	(0,016)

TABLE 2.4 Continued

TABLET	FRACTION DISSOLVED AT EACH TIME INTERVAL											
	2,6	2,8	3,0	3,2	3,4	3,6	3,8	4,0	4,2	4,4	4,6	4,8
Dolorol	0,918 (0,015)	0,930 (0,012)	0,940 (0,011)	0,949 (0,009)	0,956 (0,009)	0,962 (0,009)	0,966 (0,008)	0,971 (0,007)	-	-	-	-
Napamol	0,780 (0,014)	0,796 (0,013)	0,809 (0,010)	0,821 (0,010)	0,832 (0,010)	0,843 (0,008)	0,852 (0,008)	0,861 (0,008)	0,869 (0,008)	0,876 (0,007)	0,882 (0,007)	0,888 (0,007)
Norstan-Paracetamol	0,778 (0,021)	0,806 (0,021)	0,833 (0,019)	0,858 (0,017)	0,879 (0,015)	0,898 (0,013)	0,913 (0,012)	0,927 (0,011)	0,938 (0,009)	0,948 (0,009)	0,956 (0,008)	0,963 (0,007)
Panado	0,919 (0,038)	0,932 (0,033)	0,943 (0,028)	0,951 (0,024)	0,958 (0,020)	0,964 (0,017)	0,969 (0,015)	0,973 (0,013)	-	-	-	-
Paracetamol (Petersen B/N 32449)	0,909 (0,021)	0,921 (0,017)	0,931 (0,014)	0,940 (0,011)	0,948 (0,011)	0,954 (0,010)	0,960 (0,010)	0,965 (0,009)	-	-	-	-
Paracetamol (Petersen B/N 32978)	0,890 (0,014)	0,905 (0,012)	0,918 (0,010)	0,928 (0,009)	0,937 (0,008)	0,945 (0,007)	0,952 (0,006)	0,958 (0,005)	0,964 (0,005)	0,968 (0,004)	0,972 (0,004)	0,977 (0,003)
Paracetamol (Propan)	0,456 (0,035)	0,489 (0,037)	0,523 (0,034)	0,557 (0,037)	0,591 (0,040)	0,622 (0,042)	0,654 (0,044)	0,685 (0,050)	0,722 (0,057)	0,759 (0,060)	0,793 (0,062)	0,823 (0,059)
Repamol	0,251 (0,019)	0,267 (0,023)	0,282 (0,024)	0,297 (0,029)	0,312 (0,032)	0,326 (0,035)	0,341 (0,037)	0,355 (0,041)	0,370 (0,046)	0,385 (0,048)	0,401 (0,052)	0,417 (0,055)

TABLE 2.4 Continued

TABLET	FRACTION DISSOLVED AT EACH TIME INTERVAL											
	5,0	5,2	5,4	5,6	5,8	6,0	6,2	6,4	6,6	6,8	7,0	7,2
Dolorol	0,984 (0,004)	-	-	-	-	0,991 (0,004)	-	-	-	-	0,996 (0,003)	-
Napamol	0,894 (0,006)	-	-	-	-	0,918 (0,004)	-	-	-	-	0,935 (0,003)	-
Norstan-Paracetamol	0,970 (0,005)	-	-	-	-	0,989 (0,004)	-	-	-	-	0,996 (0,003)	-
Panado	0,986 (0,008)	-	-	-	-	0,993 (0,005)	-	-	-	-	0,996 (0,004)	-
Paracetamol (Petersen B/N 32449)	0,982 (0,007)	-	-	-	-	0,991 (0,006)	-	-	-	-	0,995 (0,004)	-
Paracetamol (Petersen B/N 32978)	0,979 (0,002)	-	-	-	-	0,990 (0,001)	-	-	-	-	0,996 (0,002)	-
Paracetamol (Propan)	0,846 (0,051)	0,867 (0,040)	0,884 (0,031)	0,898 (0,025)	0,909 (0,020)	0,918 (0,017)	0,925 (0,015)	0,932 (0,013)	0,938 (0,012)	0,943 (0,011)	0,948 (0,011)	-
Repamol	0,434 (0,058)	0,452 (0,059)	0,469 (0,060)	0,487 (0,063)	0,503 (0,065)	0,519 (0,068)	0,537 (0,072)	0,552 (0,075)	0,569 (0,079)	0,584 (0,083)	0,599 (0,087)	0,613 (0,090)

TABLE 2.4 Continued

TABLET	FRACTION DISSOLVED AT EACH TIME INTERVAL											
	7,4	7,6	7,8	8,0	8,2	8,4	8,6	8,8	9,0	9,2	9,4	9,6
Dolorol	-	-	-	0,997 (0,002)	-	-	-	-	0,999 (0,002)	-	-	-
Napamol	-	-	-	0,946 (0,005)	-	-	-	-	0,957 (0,004)	-	-	-
Norstan-Paracetamol	-	-	-	0,999 (0,001)	-	-	-	-	1,000 (0,000)	-	-	-
Panado	-	-	-	0,998 (0,003)	-	-	-	-	0,999 (0,002)	-	-	-
Paracetamol (Petersen B/N 32449)	-	-	-	0,998 (0,002)	-	-	-	-	0,999 (0,002)	-	-	-
Paracetamol (Petersen B/N 32978)	-	-	-	0,998 (0,001)	-	-	-	-	1,000 (0,000)	-	-	-
Paracetamol (Propan)	-	-	-	0,962 (0,007)	-	-	-	-	0,973 (0,006)	-	-	-
Repamol	0,627 (0,095)	0,640 (0,098)	0,653 (0,102)	0,666 (0,105)	0,678 (0,107)	0,690 (0,109)	0,701 (0,111)	0,712 (0,112)	0,725 (0,113)	0,736 (0,113)	0,748 (0,113)	0,758 (0,111)

TABLE 2.4 Continued

TABLET	FRACTION DISSOLVED AT EACH TIME INTERVAL											
	9,8	10,0	10,2	10,4	10,6	10,8	11,0	12,0	13,0	14,0	15,0	16.0
Dolorol	-	1,000 (0,001)	-	-	-	-	-	-	-	-	-	-
Napamol	-	0,965 (0,003)	-	-	-	-	0,972 (0,003)	0,978 (0,003)	0,981 (0,002)	0,985 (0,002)	0,987 (0,002)	0,989 (0,001)
Norstan-Paracetamol	-	-	-	-	-	-	-	-	-	-	-	-
Panado	-	1,000 (0,000)	-	-	-	-	-	-	-	-	-	-
Paracetamol (Petersen B/N 32449)	-	1,000 (0,000)	-	-	-	-	-	-	-	-	-	-
Paracetamol (Petersen B/N 32978)	-	-	-	-	-	-	-	-	-	-	-	-
Paracetamol (Propan)	-	0,980 (0,004)	-	-	-	-	0,986 (0,004)	0,989 (0,003)	0,993 (0,002)	0,995 (0,002)	0,997 (0,001)	0,998 (0,001)
Repamol	0,769 (0,109)	0,778 (0,110)	0,790 (0,107)	0,798 (0,108)	0,806 (0,106)	0,815 (0,104)	0,822 (0,104)	0,857 (0,094)	0,885 (0,082)	0,912 (0,068)	0,937 (0,055)	0,958 (0,039)

TABLE 2.4 Continued

TABLET	FRACTION DISSOLVED AT EACH TIME INTERVAL									
	17,0	18,0	19,0	20,0	21,0	22,0	23,0	24,0	25,0	26,0
Dolorol	-	-	-	-	-	-	-	-	-	-
Napamol	0,991 (0,002)	0,993 (0,002)	0,994 (0,002)	0,995 (0,001)	0,997 (0,001)	0,997 (0,001)	0,998 (0,002)	0,999 (0,002)	1,000 (0,001)	-
Norstan-Paracetamol	-	-	-	-	-	-	-	-	-	-
Panado	-	-	-	-	-	-	-	-	-	-
Paracetamol (Petersen B/N 32449)	-	-	-	-	-	-	-	-	-	-
Paracetamol (Petersen B/N 32978)	-	-	-	-	-	-	-	-	-	-
Paracetamol (Propan)	0,999 (0,001)	1,000 (0,000)	-	-	-	-	-	-	-	-
Repamol	0,969 (0,034)	0,979 (0,025)	0,985 (0,018)	0,990 (0,013)	0,994 (0,008)	0,996 (0,006)	0,997 (0,004)	0,998 (0,003)	0,999 (0,003)	1,000 (0,001)

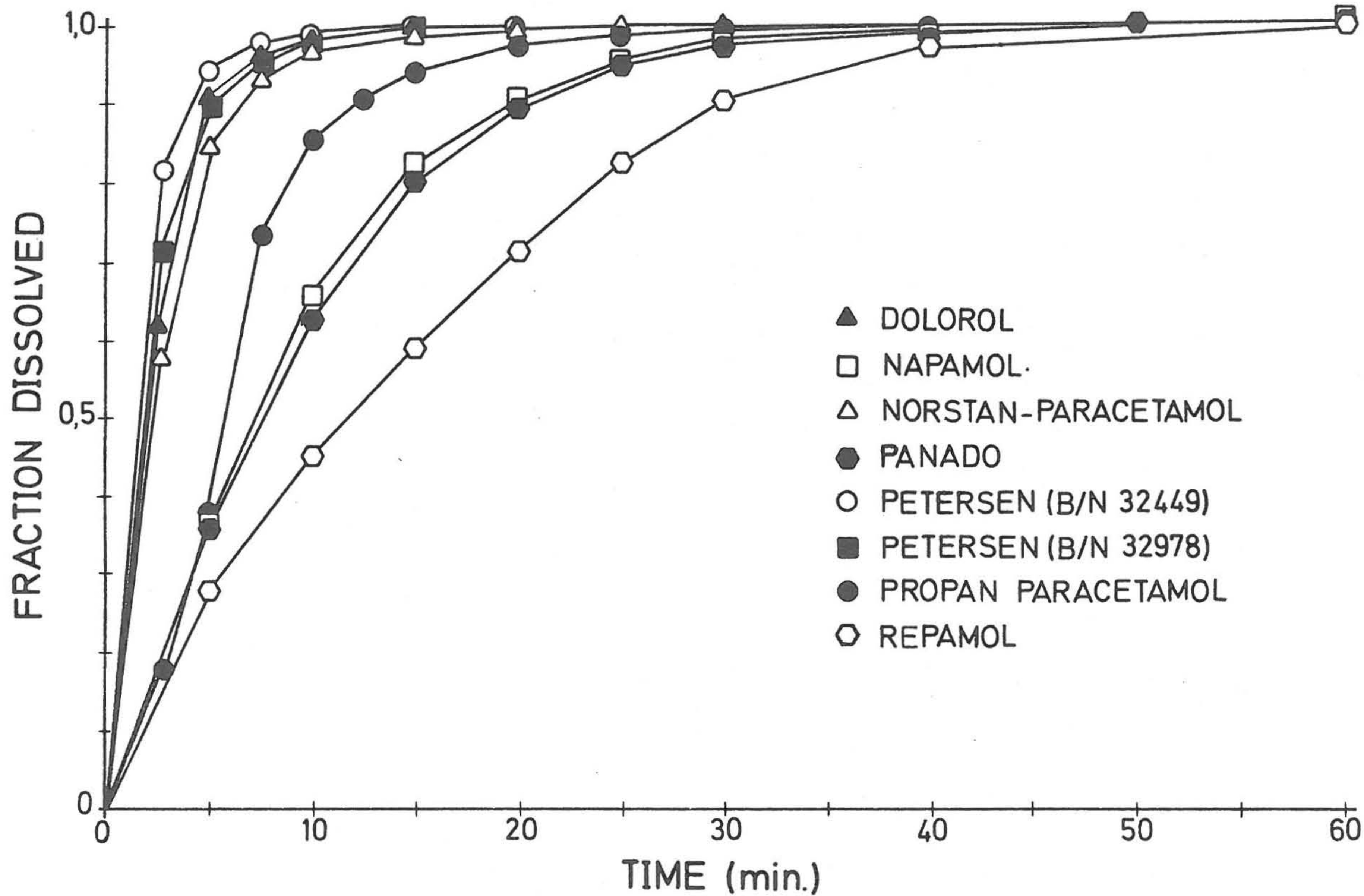


FIGURE 2.5 The dissolution curves of eight lots of paracetamol tablets, determined manually in a USP Rotating Basket Apparatus.

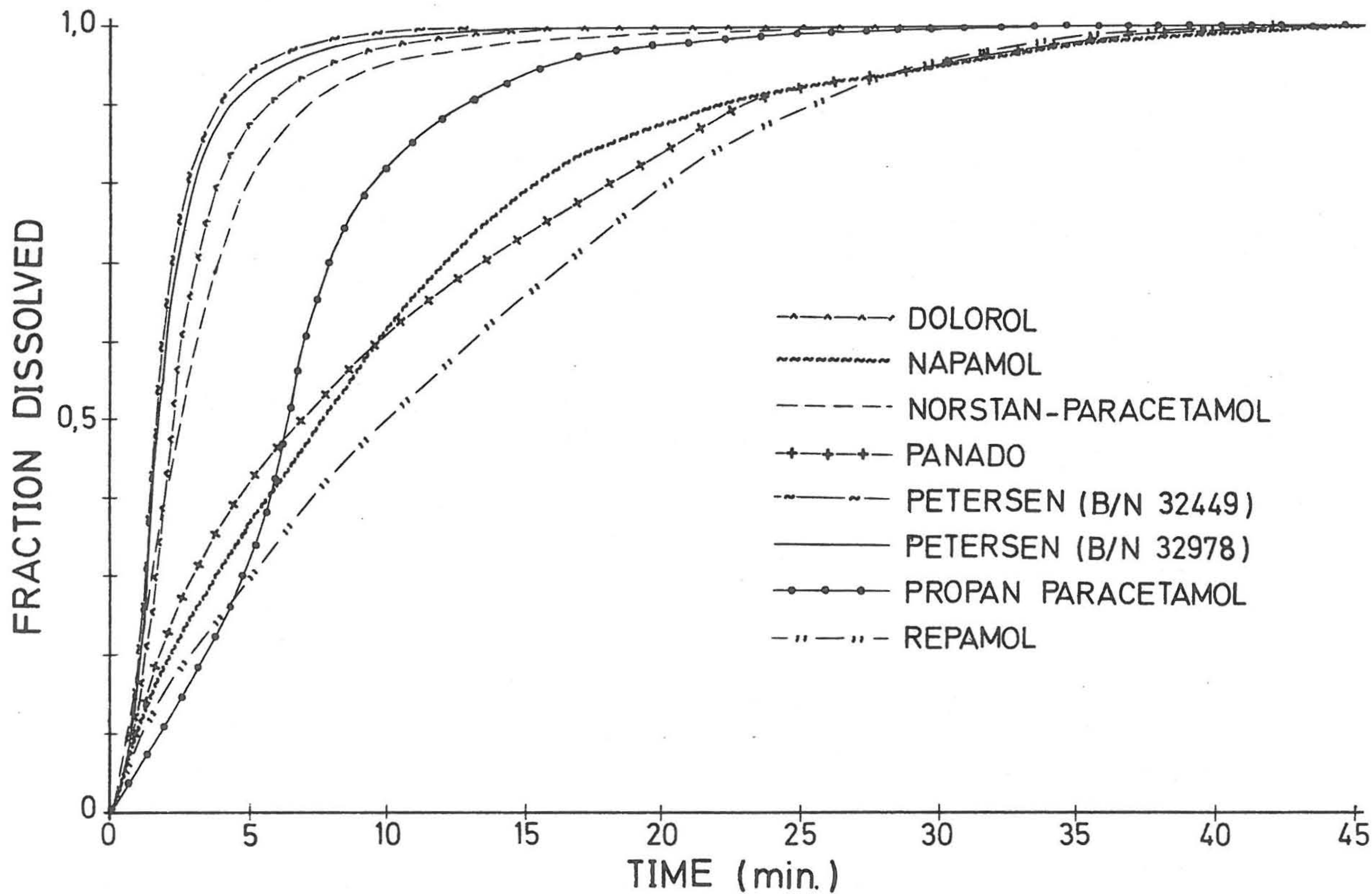


FIGURE 2.6 The dissolution curves of eight lots of paracetamol tablets, determined in an automated USP Rotating Basket Apparatus.

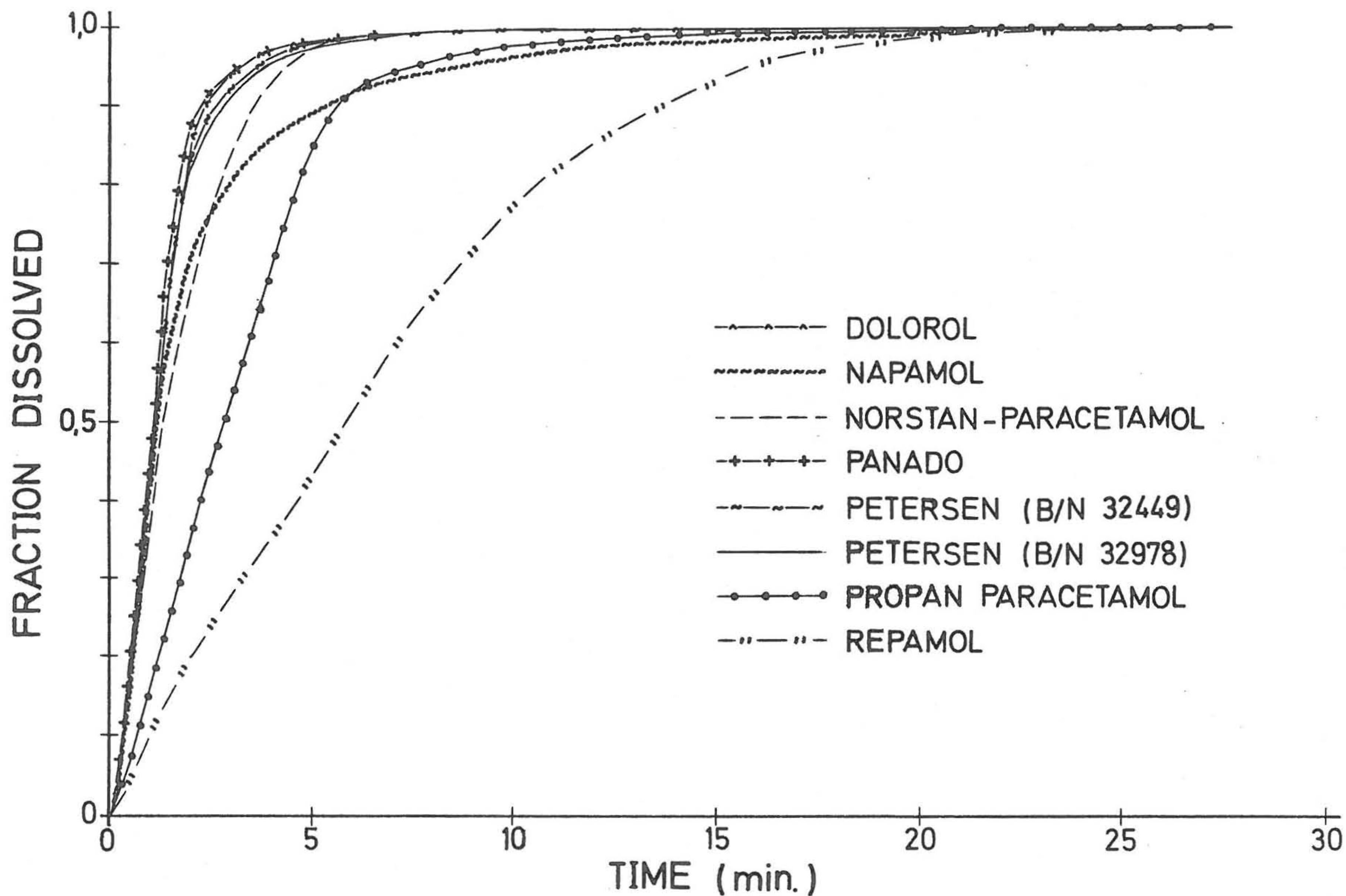


FIGURE 2.7 The dissolution curves of eight lots of paracetamol tablets, determined in an automated Stationary Basket-Rotating Paddle Apparatus.

with sporadic segmentation and another trace obtained with regular segmentation.

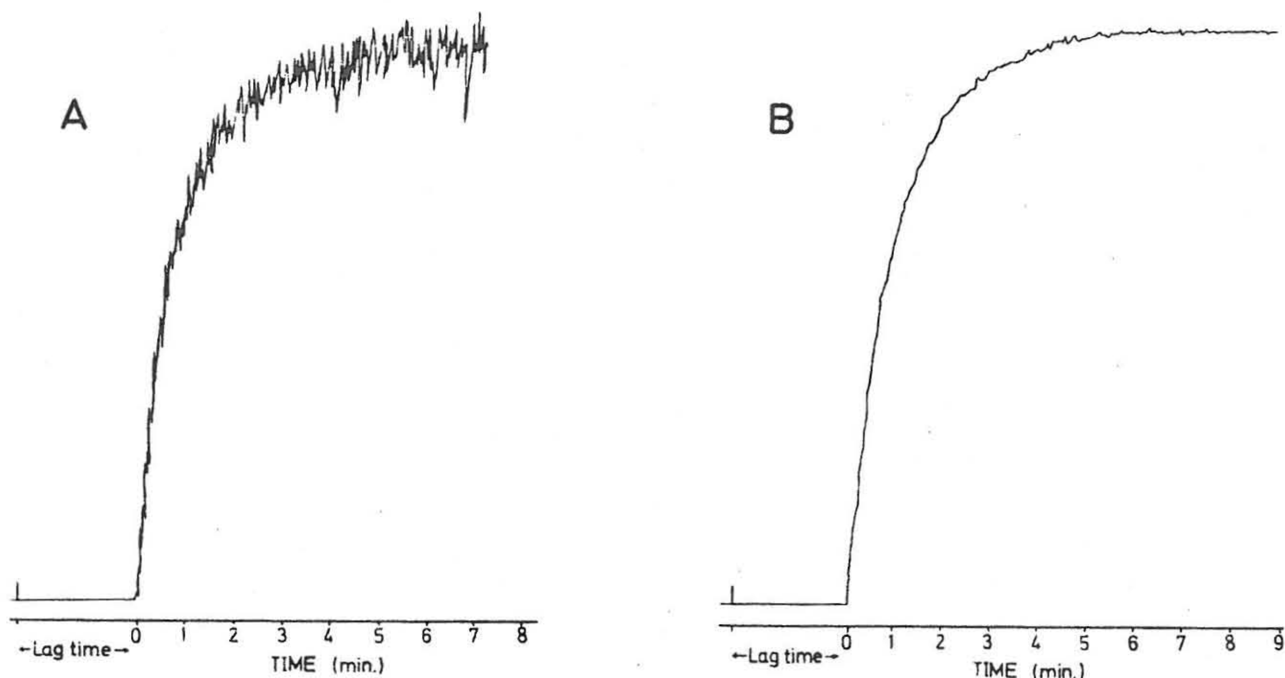


FIGURE 2.8 Dissolution traces obtained with sporadic segmentation (A) and regular segmentation (B) of the fluid stream within the transmission tubing.

The debubbling unit was found to require a positive pressure at the inlet of the flow cell and a negative pressure at the outlet of the flow cell to function efficiently. This was achieved by pumping 2,0 ml/min. of diluted sample from the outlet of the flow cell. Excess sample arriving at the flow cell, together with air bubbles, ran to waste under their own pressure. (See Figure 2.3 for the flow diagram.)

The sample volume removed from the dissolution vessel was so small (0,16 ml/min.), that less than 5 ml was generally removed during a given determination. Since this represented 0,3 and 0,5% of the total volume of dissolution medium in the stationary basket and rotating basket methods respectively, it was not considered necessary to replace fluid.

The manner in which the data points for the automated dissolution profiles were calculated ignored differences between tablets that were due to variations in tablet content of active medicament. The manual curves were also adjusted to one hundred percent. This reduced within group variation and allowed dissolution trends to stand out more clearly. (In all cases, it was established that the actual results obtained, agreed well with the label strength of the tablet.)

CHAPTER 3

METHODS OF EXPRESSING DISSOLUTION RATE DATA

3.1 INTRODUCTION

The qualitative interpretation of dissolution rate data has limited usefulness because of the difficulty in assessing differences between profiles and establishing standards with which the profiles have to comply. The importance of transforming dissolution rate data into quantitative parameters that will give a meaningful and reliable indication of the bioavailability of the formulation has therefore long been recognized.

The amount of drug dissolved in a given time interval has been used to correlate *in vivo* and *in vitro* results.[104,108,132-134] The time interval used is determined empirically by comparing dissolution rate data and blood or urine level data in order to see whether a relationship can be found between the two. Once a relationship has been established and confirmed, tablets may be assessed by means of dissolution rate data alone. There are however a number of reports in the literature of tablets which produced unacceptable dissolution profiles yet yielded perfectly acceptable blood levels of the active ingredient.[135-137] This implies that caution should be exercised in the utilization of this *in vitro* parameter as a measure of bioavailability.

The time taken for fifty percent of the dose to dissolve ($t_{50\%}$) has also been quoted frequently in the literature and has been correlated with blood level data.[104,138] The $t_{50\%}$ gives an indication of the rate at which the tablet dissolves and can easily be obtained directly from the dissolution curve. There are nonetheless reservations about whether a single point on a dissolution profile can characterize the entire curve adequately.[139]

Various mathematical functions have been employed to describe the dissolution profiles more fully.[139-142] Data have been interpreted according to first order kinetics and the cube root law, but both of these expressions have limited applicability to the dissolution profiles of formulated products. The lack of fit is due to the fact that the functions are based on idealized, monodisperse systems which are rarely found in practice.[143,146]

Langenbucher proposed that the Weibull distribution function would be a useful means of summarizing dissolution rate data, since it can be applied to all common dissolution curves including profiles with a pronounced sigmoidal shape.[144] The Weibull function is a general mathematical expression which can be written in the following forms:

$$m = 1 - \exp\left(-\frac{(t-Ti)^b}{a} \right) \qquad (3.1)$$

$$\log(-\ln(1-m)) = b.\log(t-Ti) - \log a \qquad (3.2)$$

where 'm' is the fraction dissolved at time 't'. 'Ti', the location parameter, gives an indication of the lag time due to disintegration, 'a', the scale parameter, gives an indication of the time scale of the dissolution process, and 'b', the shape parameter, describes the general shape of the dissolution curve. If 'b' equals 1 then the curve is exponential, if 'b' is greater than 1 then the curve is S-shaped and if 'b' is less than 1 then the initial slope of the curve is too steep to be consistent with an exponential.

In addition to the above parameters, one can obtain a fourth parameter that is comparable to the $t_{50\%}$. This is known as the dissolution time, 'Td', and is defined as the time at which $-\ln(1-m) = 1$. At this point 63,2% of the dose has dissolved.

Data can be fitted to equation 3.1 by means of a suitable non-linear curve fitting technique.[145] If the linear form of the equation is used (equation 3.2), the scale and the shape parameters can be obtained from the Y-intercept and slope of a log/log plot of -ln(1-m) *versus* time, respectively. The location parameter can however only be found indirectly by trying various time shifts until one finds the one that gives the best linearization of the data. This can either be assessed graphically or by means of linear regression.

The main advantage of the Weibull distribution function is its ability to describe a large number of dissolution curves. It is however an empirical equation and this tends to limit the kinetic significance of the parameters that are obtained from it.

A kinetically based equation has been presented by Pedersen et al., which is able to describe the intrinsic dissolution properties of a drug under sink and non-sink conditions.[146] The equation is applicable to both monodisperse and polydisperse systems. The intrinsic dissolution rate, the particle distribution effect, the disintegration effect and the amount of drug available for dissolution can be evaluated directly from fraction dissolved versus time data. A computer program for the above transform could not be compiled in these laboratories, as a special subroutine is required to enable computation.[147] The dissolution rate data from this study could therefore not be fitted to the kinetic equation.

Parameters from the dissolution rate data are listed in Tables 3.1, 3.2 and 3.3. The fraction of the dose dissolved at various time intervals is presented in Tables 2.2, 2.3 and 2.4 (page 24 to 36).

3.2 THE STATISTICAL ANALYSIS OF RESULTS

Results from the automated dissolution apparatuses were evaluated by means of one way analysis of variance.[148] The tablets giving rise to significant differences were identified by the application of the Scheffé test.[149] Results from the latter analysis are shown in Tables A.1 and A.2. (See Appendix 1 and 2). The 95% confidence level was employed in the statistical analysis.

3.3 RESULTS AND DISCUSSION

3.3.1 The USP Rotating Basket Apparatus

The following trends were noted when the cumulative fraction dissolved was investigated. The four rapidly dissolving tablets (Dolorol, Norstan-Paracetamol and both batches of Petersen paracetamol) did not differ significantly from one another during the first seven minutes. The four more slowly dissolving tablets did not differ significantly from one another either. Each tablet in the rapidly dissolving group however differed significantly from each of the tablets in the other group. The situation remained essentially the same from the 8 to the 13 minute readings. Propan paracetamol however ceased to differ significantly from the rapidly dissolving tablets in this period and became significantly different from Repamol. After the 13 minute reading Repamol alone

differed significantly from the rapidly dissolving tablets. There were no significant differences between any of the other tablets.

The results for the $t_{50\%}$ and the dissolution time (Td) were almost identical. Repamol differed significantly from all of the rapidly dissolving tablets, Napamol differed significantly from both batches of Petersen paracetamol and Panado differed significantly from both batches of Petersen paracetamol in the case of the dissolution time and one batch of Petersen paracetamol (B/N 32449) in the case of the $t_{50\%}$.

For the scale parameter (a), Repamol was found to differ significantly from all of the rapidly dissolving tablets with the exception of Dolorol, and Propan paracetamol was found to differ significantly from all of the tablets with the exception of Repamol.

There were no significant differences between tablets when the location parameter (Ti) was compared and Propan paracetamol was the only tablet to exhibit significant differences when the shape parameter (b) was compared. In the latter case Propan paracetamol differed from all of the tablets with the exception of Dolorol.

3.3.2 The Stationary Basket-Rotating Paddle Apparatus

Analysis of the cumulative fraction dissolved data showed that Repamol differed significantly from essentially all of the other tablets in the first 15 minutes, beyond which time none of the tablets differed significantly from one another. Propan paracetamol only differed significantly from the other tablets in the first 5 minutes. Apart from a few instances within the first 5 minutes, when Norstan-Paracetamol and Napamol differed significantly from Dolorol, Panado and both batches of Petersen paracetamol, the other tablets did not differ significantly from one another.

The results from the $t_{50\%}$, the scale parameter (a) and the dissolution time (Td) showed identical trends. i.e. Repamol and Propan paracetamol differed significantly from all of the other tablets and were the only two tablets to exhibit significant differences. Napamol differed significantly from all of the other tablets when the shape parameter (b) was compared and in the case of the location parameter (Ti), Repamol differed significantly from all of the tablets with the exception of Propan paracetamol, and Napamol differed significantly from the latter tablet.

TABLE 3.1 Parameters obtained from the dissolution profiles determined in the manual USP rotating basket apparatus.

Each reading represents the average of six determinations. The coefficient of variation appears below each mean in parentheses.

TABLET NAME	$t_{50\%}$	PARAMETERS FROM THE WEIBULL FUNCTION			
		a	b	Ti	Td
Dolorol	2,18 (34,47)	1,18 (50,40)	0,68 (34,60)	1,13 (92,12)	2,23 (43,64)
Napamol	7,97 (52,45)	24,75 (139,61)	1,25 (16,40)	0,91 (121,99)	9,82 (46,61)
Norstan-Paracetamol	2,20 (15,06)	1,33 (49,10)	0,66 (23,51)	1,36 (54,11)	2,77 (14,63)
Panado	8,09 (20,36)	21,05 (76,04)	1,21 (30,79)	0,90 (190,27)	9,94 (25,98)
Paracetamol (Petersen B/N 32449)	1,55 (6,31)	0,66 (29,96)	0,48 (25,09)	1,30 (58,66)	1,74 (28,04)
Paracetamol (Petersen B/N 32978)	1,89 (39,02)	1,20 (31,60)	0,71 (17,56)	0,69 (127,22)	1,94 (50,30)
Paracetamol (Propan)	5,88 (13,43)	6,48 (59,71)	1,05 (10,15)	1,41 (32,67)	6,80 (20,02)
Repamol	12,13 (28,11)	32,00 (66,82)	1,24 (18,12)	0,28 (244,95)	14,62 (19,66)

TABLE 3.2 Parameters obtained from the dissolution profiles determined in the automated USP rotating basket apparatus.

Each reading represents the average of four determinations. The coefficient of variation appears below each mean in parentheses.

TABLET NAME	$t_{50\%}$	PARAMETERS FROM THE WEIBULL FUNCTION			
		a	b	Ti	Td
Dolorol	2,21 (13,17)	4,23 (27,49)	1,24 (7,19)	0,14 (16,68)	3,26 (13,28)
Napamol	8,20 (26,90)	10,56 (26,80)	1,06 (11,27)	0,03 (200,00)	9,51 (28,94)
Norstan-Paracetamol	2,40 (10,04)	3,50 (12,66)	1,06 (5,32)	0,16 (12,68)	3,43 (7,03)
Panado	8,01 (52,11)	9,90 (44,40)	1,04 (2,36)	0,16 (13,59)	9,28 (42,83)
Paracetamol (Petersen B/N 32449)	1,55 (10,40)	2,40 (17,56)	1,21 (3,69)	0,15 (12,94)	2,19 (11,80)
Paracetamol (Petersen B/N 32978)	1,80 (16,76)	2,99 (26,35)	1,23 (5,67)	0,14 (12,10)	2,53 (16,69)
Paracetamol (Propan)	6,33 (7,19)	22,61 (25,85)	1,48 (7,38)	0,04 (88,59)	8,14 (7,73)
Repamol	10,46 (31,30)	13,20 (27,64)	1,06 (7,16)	0,07 (120,14)	11,42 (23,79)

TABLE 3.3 Parameters obtained from the dissolution profiles determined in the automated stationary basket-rotating paddle apparatus.

Each reading represents the average of four determinations. The coefficient of variation appears below each mean in parentheses.

TABLET NAME	$t_{50\%}$	PARAMETERS FROM THE WEIBULL FUNCTION			
		a	b	Ti	Td
Dolorol	1,11 (11,57)	1,36 (18,65)	1,15 (7,74)	0,15 (8,75)	1,44 (13,48)
Napamol	1,11 (8,42)	1,49 (9,25)	0,73 (6,40)	0,19 (3,20)	1,90 (8,58)
Norstan-Paracetamol	1,34 (17,54)	2,19 (23,26)	1,30 (11,57)	0,16 (16,57)	1,95 (11,84)
Panado	1,00 (24,76)	1,29 (36,60)	1,14 (10,42)	0,15 (8,78)	1,37 (26,92)
Paracetamol (Petersen B/N 32449)	1,01 (11,97)	1,28 (17,13)	1,10 (3,44)	0,14 (6,82)	1,38 (14,04)
Paracetamol (Petersen B/N 32978)	1,13 (11,02)	1,45 (16,40)	1,15 (9,53)	0,15 (6,96)	1,52 (10,70)
Paracetamol (Propan)	2,87 (7,50)	5,38 (11,60)	1,35 (1,85)	0,07 (101,17)	3,54 (7,91)
Repamol	5,92 (13,79)	11,46 (10,50)	1,26 (9,50)	0,04 (100,00)	7,05 (16,26)

3.3.3 General Discussion

The rotating basket apparatus has been reported to lack reproducibility.[74,126] The present study tends to confirm this finding as the coefficients of variation listed in Table 3.2 (rotating basket apparatus) are generally greater than those listed in Table 3.3 (stationary basket-rotating paddle apparatus).

The parameters obtained from the Weibull distribution function were found to differ markedly when dissolution rate data obtained from the manual and the automated USP rotating basket assemblies were fitted to the equation. (See Tables 3.1 and 3.2). This was true for data that had been processed according to both the linear and the non-linear form of the equation. The above differences could not be ascribed to large changes in the characteristics of the dissolution curves, as the profiles obtained in the manual method were very similar to those obtained in the automated method. Subsequent investigations indicated that the parameters obtained from the Weibull function depend upon the number of data points used for the fit. The above discrepancies would therefore appear to be due to the fact that the manual dissolution curves were described by far fewer points than the automated dissolution curves. As a result of the above findings, only the data that had been obtained from the automated dissolution methods were assessed statistically.

CHAPTER 4

THE ANALYSIS OF PARACETAMOL AND ITS METABOLITES IN BIOLOGICAL FLUIDS

4.1 METHODS AVAILABLE FOR THE DETERMINATION OF PARACETAMOL IN BIOLOGICAL FLUIDS

The first reported methods of analysing paracetamol in biological fluids were colorimetric. They generally involve extraction into ether, returning the paracetamol to alkali, and acid hydrolysis to p-aminophenol. The p-aminophenol is coupled to compounds such as alpha-naphthol,[150,151] phenol in the presence of sodium hypobromite[150,152] or vanillin,[153] to yield a coloured product which can be assayed colorimetrically. In order to determine total paracetamol (free and conjugated) acid hydrolysis to p-aminophenol is performed before the extraction step, as conjugates are not readily extracted into organic solvents because of their polar nature.[150] Total paracetamol has also been determined in urine by the formation of an indophenol dye without an extraction step.[62]

Another colorimetric approach involves the reaction of paracetamol with diphenylpicrylhydrazyl to form a yellow diphenylpicrylhydrazine[151] and a third approach involves the reaction of paracetamol with nitrous acid to form 2-nitro-4-acetamidophenol, which can be measured by its orange-red colour in alkaline solutions.[34,154,155]

The above methods have tended to lose favour because of their lengthiness and lack of sensitivity, as well as the fact that they do not differentiate between the metabolites of paracetamol.[156,157]

A differential absorbance technique has been applied to the analysis of paracetamol, following extraction into a suitable organic solvent and re-extraction into an aqueous sodium bicarbonate solution.[34,151] The main advantage of this method is its rapidity and simplicity. There is however a strong likelihood of interference by other drugs.[156]

Gas chromatographic (GC) methods were developed in order to increase the sensitivity and specificity of the analysis of paracetamol.

Chromatography of the drug itself was found to produce peaks with pronounced tailing and was subject to adsorption losses at low concentrations.[156-158] Various derivatives were therefore formed in order to overcome these problems. These included trimethylsilyl,[43,156,159] acetyl,[160-163] O-heptyl-N-methyl,[34] N,O-dimethyl[164] and O-butyryl[165] derivatives. In order to determine total paracetamol in a sample, glucuronide and sulphate groups were removed enzymatically before derivatization.[43,156]

No matter which derivative is formed, the main drawback with the GC methods is the complex and time-consuming sample preparation that they entail and the fact that they do not differentiate between the metabolites of paracetamol. The methods involving silylation of paracetamol have also been criticized because the derivative is unstable,[156,160,161] silylation is not always complete,[159,161] contaminating compounds may give rise to interfering peaks[159,160] and the flame ionization detector may become contaminated with silica deposits.[160]

High pressure liquid chromatography (HPLC) has become the method of choice in recent years. Its main advantage over GC methods is the fact that paracetamol can be determined without the formation of a derivative and sample preparation is therefore relatively simple. Many of the reported methods include extraction into an organic solvent and can only be used to monitor unchanged paracetamol.[166-172] There are however also methods in the literature in which samples can be injected without this step, after protein precipitation and centrifugation.[42,48,173-176] The latter methods are therefore suitable for the simultaneous determination of paracetamol and its metabolites.

4.2 THE DEVELOPMENT OF A METHOD FOR ANALYSING PARACETAMOL AND ITS METABOLITES IN BIOLOGICAL FLUID BY HPLC

HPLC was chosen as the method of analysis for this study because the technique is sufficiently sensitive to detect paracetamol at therapeutic concentrations, both the free drug and its metabolites can be determined simultaneously, and the relatively simple and rapid sample preparation that it entails lends itself to the analysis of a large number of samples.

4.2.1 The Column Packing Material

An anion exchange column has been applied to the determination of paracetamol and its metabolites in urine.[177] The analysis was however very lengthy and took 21 to 42 hours for each determination. The analysis of phenacetin and its metabolites on the same type of column was also a lengthy determination.[178]

A cation exchange column has been used to determine free paracetamol in serum[166,179] and urine.[166] The elution time was less than 5 minutes in the one report, but a polyamide column had to be used to determine paracetamol in the urine samples as the drug could not be adequately resolved from uric acid on the cation exchange column.[166]

Silica columns have also been used to determine free paracetamol in serum[167,171] and urine.[167] Elution times were acceptably short, but the silica column is unlikely to be suitable for the determination of unchanged paracetamol together with its more polar metabolites as the determination is likely to become lengthy.

The most commonly used column for the analysis of paracetamol alone[168-170,172] and together with its metabolites[42,48,173-176] has been the reverse phase octadecyl silane (C_{18}) column. It is a relatively stable column provided that the guidelines recommended by the manufacturer are followed and as a result of its widespread use much information is available regarding the compatability of different solvents with the packing material.[180] For the above reasons a reverse phase C_{18} column was chosen for this study.

4.2.2 The Detector

Paracetamol and its metabolites absorb in the uv range and spectrophotometric detection was therefore utilized. An electrochemical detector could also have been used,[166,170] but since 1 ml samples were available for the analysis, the increased sensitivity that the latter affords was not necessary.

4.2.3 The Mobile Phase

Methanol-water-formic acid (20: 80: 0,1 v/v) was initially chosen as the mobile phase. The acid was included in the mixture because

reports indicate that the peaks are poorly shaped and the metabolites are not adequately retained in its absence.[174] The system was run at ambient temperatures ranging from 23°C to 34°C and at a flow rate of 1,0 ml/min. Under these conditions the paracetamol and paracetamol glucuronide peaks were well resolved and symmetrical. The resolution of the paracetamol cysteine and paracetamol sulphate peaks from one another was however extremely poor and their retention times were identical at higher temperatures. (See Figure 4.1).

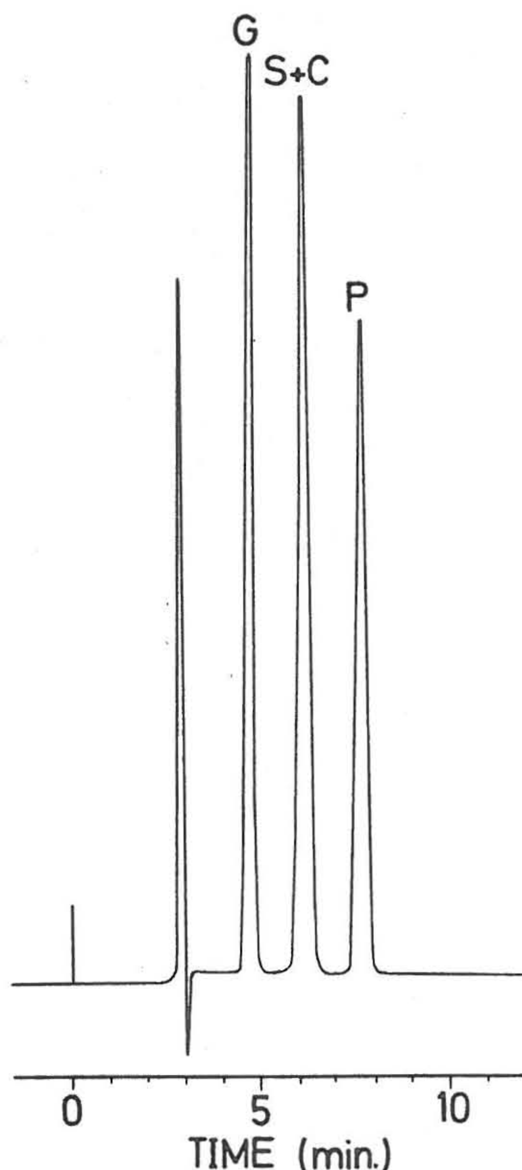


FIGURE 4.1. A chromatogram of paracetamol (P) and its glucuronide (G), cysteine (C) and sulphate (S) metabolites.
MOBILE PHASE: methanol-water-formic acid
(20: 80: 0,1 v/v) TEMP. (ambient): 28°C

This system could have been used to analyse the serum samples from the bioavailability trial without any further alterations since free paracetamol, the compound of interest, was well resolved and a linear relationship was shown to exist between the peak height ratios (paracetamol/internal standard) and the serum concentrations of the drug in the range 1 to 100 mcg/ml (r = 0,99997). The monitoring of the amount of free drug excreted in the urine does not however provide sufficient information about variations in the bioavailability of different dosage forms, as biotransformation is the major route of paracetamol elimination and only 3 to 4% of a given dose is excreted unchanged.[30] Attempts were therefore made to resolve the sulphate and cysteine peaks so that the urine could be assayed for total drug.

Flow rate changes had little or no effect upon the separation and temperature control was therefore introduced. As expected from the earlier indications, increasing the column temperature did not improve resolution. Decreasing the column temperature gave slightly better resolution, but even at the lowest column temperature that the cooling system was capable of producing(13°C), separation of the cysteine and sulphate peaks remained unsatisfactory.

The methanol-water ratio of the mobile phase was changed to 15: 85 v/v. At a given temperature, separation with the new mobile phase was better than before and, as before,it improved slightly as the column temperature decreased. Even under these conditions however, satisfactory separation was not achieved. (See Figure 4.2.)

The possibility of determining the total amount of paracetamol excreted in the urine by acid hydrolysis of the drug and its conjugates to p-aminophenol and subsequent analysis of the p-aminophenol by means of HPLC was investigated. The p-aminophenol proved to be very unstable and this approach was therefore not considered feasible.

An attempt was then made to achieve the separation by increasing the length of column through which the sample had to pass. This was accomplished by placing a Radial Compression Separation System (RCSS)

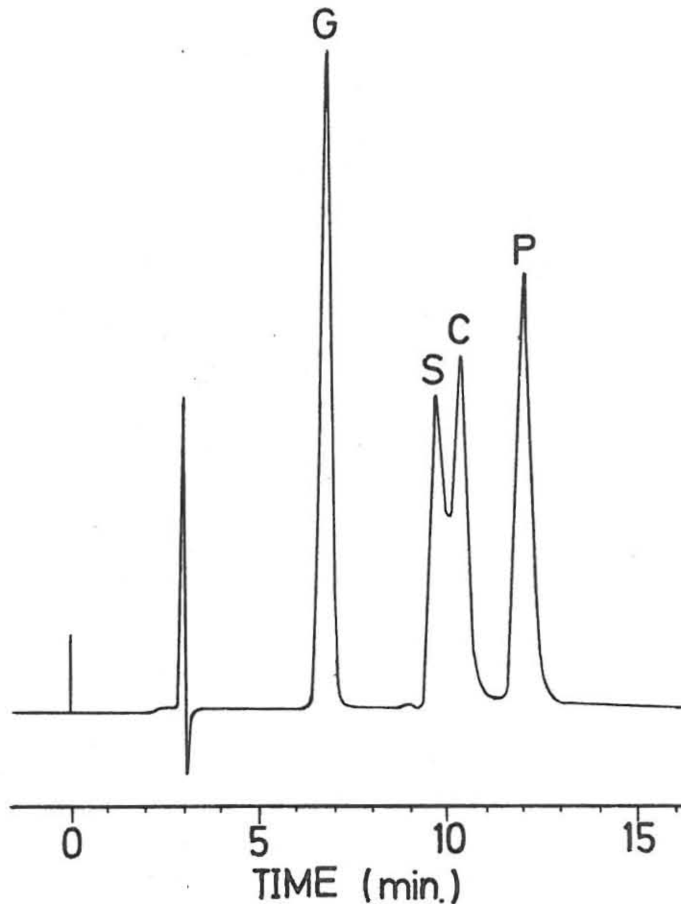


FIGURE 4.2 A chromatogram of paracetamol (P) and its glucuronide (G) cysteine (C) and sulphate (S) metabolites.

MOBILE PHASE: methanol-water-formic acid (15: 85: 0,1 v/v). TEMP.: 13°C.

fitted with a Radial-PAK A cartridge (Waters Associates) in series with the C_{18} column. The incorporation of an RCSS into the system was considered preferable to that of a second, conventional column, since the latter arrangement was likely to have generated a prohibitive column pressure. At a flow rate of 1,0 ml/min. the RCSS raised the column pressure from 1000 to 1400 psi. In addition to the above, the chromatographic characteristics of the two types of columns differ markedly in spite of the fact that they contain the same packing material and it was hoped that this would aid separation. A typical chromatogram is shown in Figure 4.3.

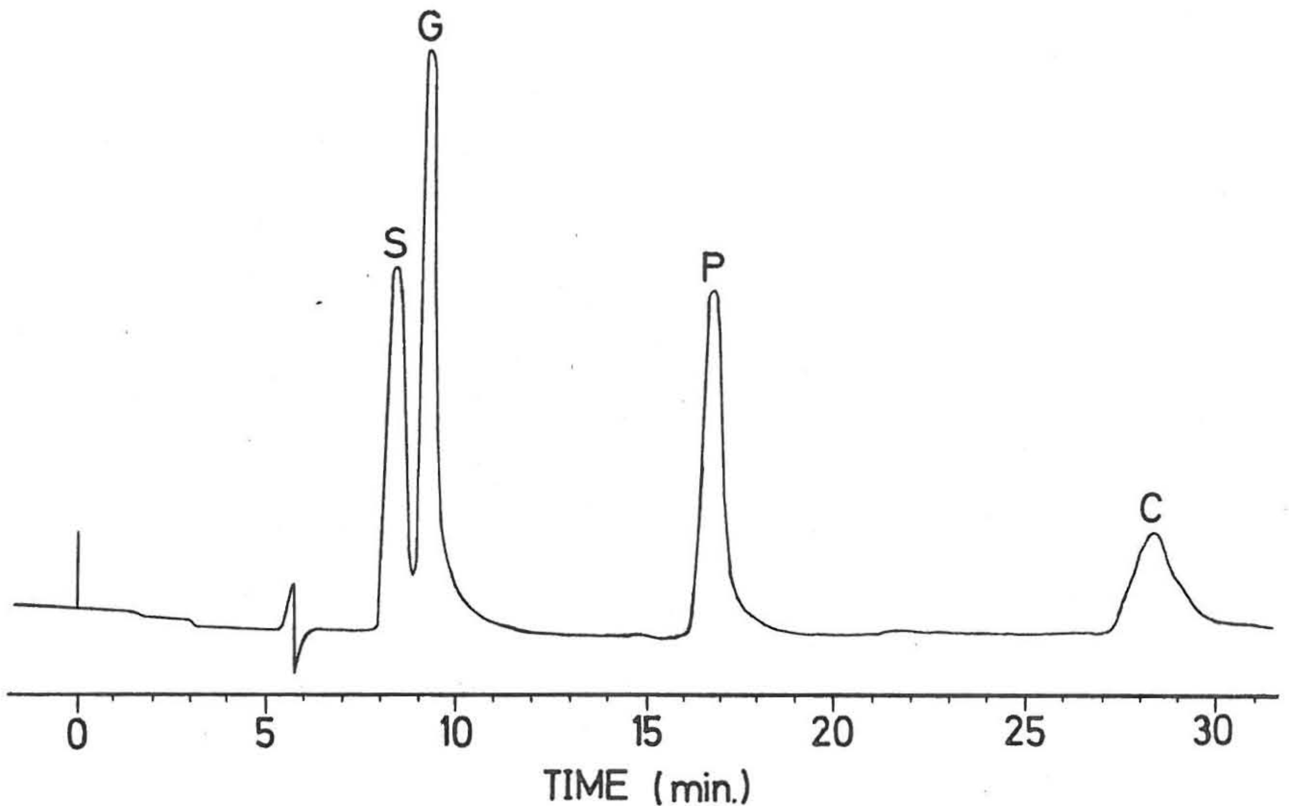


FIGURE 4.3 A chromatogram of paracetamol (P) and its glucuronide (G), cysteine (C) and sulphate (S) metabolites.

MOBILE PHASE: methanol-water-formic acid (20: 80: 0,1 v/v). COLUMN: an RCSS fitted with a Radial-PAK A cartridge, has been placed in series with a μ Bondapak C_{18} column.

The four compounds had entirely different elution patterns under the new conditions and peaks were not as sharp and symmetrical as those obtained with the single, conventional column. The paracetamol sulphate and paracetamol cysteine peaks were widely separated, but the paracetamol sulphate and paracetamol glucuronide peaks were now close together. The latter compounds were however sufficiently well resolved to allow for quantitation. In spite of the fact that this system was capable of detecting all four of the compounds simultaneously, its lengthiness made it unsuitable for the analysis of large numbers of samples. The RCSS was therefore removed and the effect of altering the composition of the mobile phase was again investigated with a single, conventional, C_{18} column.

When the methanol content of the mobile phase was replaced with acetonitrile to yield a solvent system comprising of acetonitrile-water-formic acid (10: 90: 0,1 v/v), good separation of all of the compounds was achieved. (See Figure 4.4.) The peaks were sharp and symmetrical, baseline resolution occurred between each peak and all four compounds eluted within 9 minutes. After ascertaining that there was no interference from serum and urine peaks, the above mobile phase was adopted for the study.

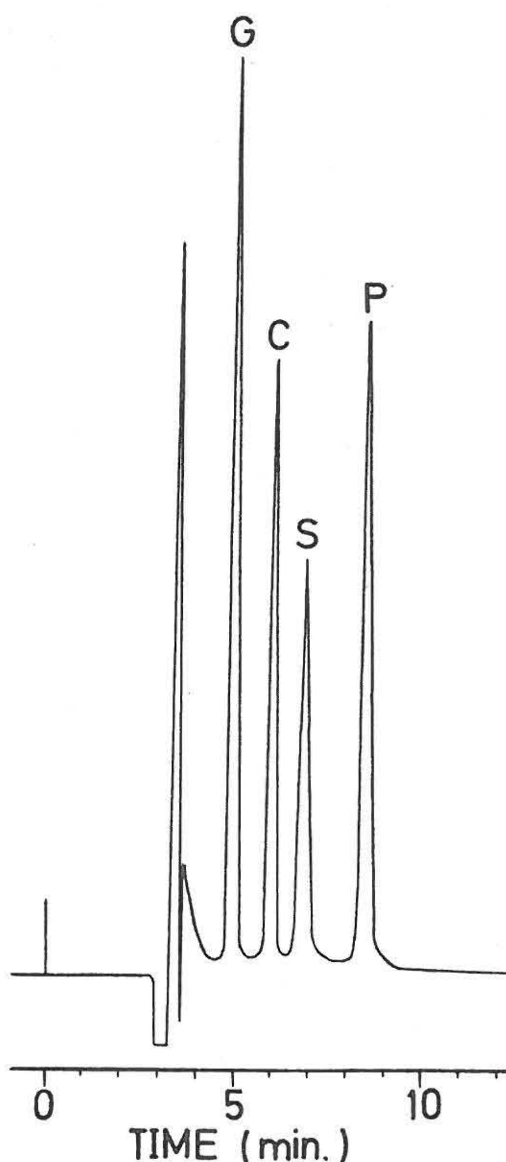


FIGURE 4.4 A chromatogram of paracetamol (P) and its glucuronide (G), cysteine (C) and sulphate (S) metabolites.

MOBILE PHASE: acetonitrile-water-formic acid (10: 90: 0,1 v/v). TEMP.: 22°C.

4.2.4 The Internal Standard

A number of compounds were investigated during the search for a suitable internal standard. Anhydrous theophylline was found to produce a sharp, symmetrical peak which eluted soon after paracetamol at 10,5 minutes. Since solutions of theophylline are relatively stable over the entire pH range,[181] this compound was chosen as the internal standard. The use of a commercially available drug as an internal standard is generally not ideal, as there is always a risk that blood and urine samples will contain the drug. Under the controlled conditions of the *in vivo* trial, theophylline and other xanthines could however be excluded from the diet of the volunteers and the chances of interference were therefore minimal.

4.2.5 The Preparation of Serum Samples for Analysis

Serum samples were prepared for injection by protein precipitation and centrifugation. In order to precipitate the protein, 1,0 ml of 3,5% aqueous perchloric acid was added to 1,0 ml of serum in a Vacutainer tube. The contents of the tube were mixed on a vortex mixer and allowed to stand for approximately 5 minutes to ensure complete precipitation of the protein before being centrifuged at 3000 rpm for 10 minutes. The clear supernatant liquid was injected directly onto the column.

Perchloric acid was chosen in preference to trichloroacetic acid as protein precipitant because the former produced a "cleaner" sample. The internal standard was dissolved in the aqueous perchloric acid (50 mcg/ml) and was added to the serum sample during the protein precipitation step.

4.2.6 The Preparation of Urine Samples for Analysis

Sample preparation involved the addition of 1,0 ml of methanol, 2,0 ml of distilled water and 4,0 ml of 3,5% aqueous perchloric acid containing internal standard (2500 mcg/4 ml), to 1,0 ml of urine in a Vacutainer tube. The contents of the tube were mixed on a vortex mixer and centrifuged at 3000 rpm for 10 minutes. The clear supernatant liquid was injected directly onto the column.

The addition of perchloric acid to the urine samples produced no precipitation. It was however not omitted as its presence was found to be necessary for a well-shaped paracetamol sulphate peak.
During the developmental stages of the HPLC analysis, an equal volume of 3,5% aqueous perchloric acid had been added to all of the aqueous samples in order to mimic the conditions that would be encountered in the serum analysis as closely as possible. In the absence of perchloric acid, paracetamol sulphate peaks were found to develop a shoulder. This occurred in both spiked aqueous samples and urine samples that had been collected after the ingestion of paracetamol. (See Figure 4.5).

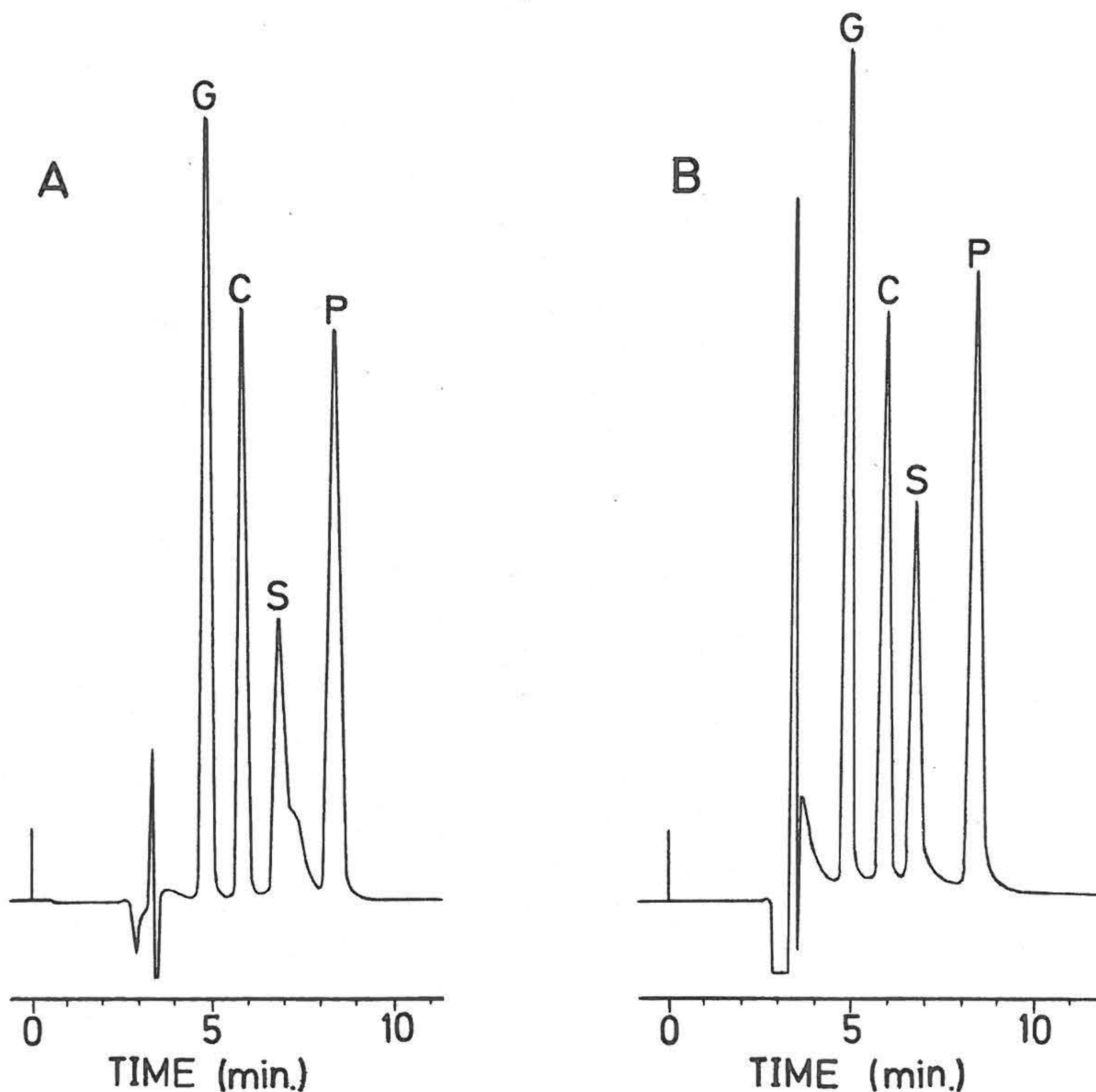


FIGURE 4.5 A chromatogram of paracetamol (P) and its glucuronide (G), cysteine (C) and sulphate (S) metabolites.

Sample (a) is aqueous; sample (b) contains perchloric acid.

The addition of methanol to the urine samples produced a precipitate in many cases and yielded a "cleaner" sample. The distilled water was added to bring the final volume of the sample to 8,0 ml, as this volume brought the sample into the correct concentration range for analysis.

4.3 THE CALIBRATION CURVES

4.3.1 The Calibration Curve of Paracetamol in Serum

Blank serum was spiked with paracetamol in the concentration range of 1 to 100 mcg/ml. This was accomplished by making up a methanolic solution of the drug, pipetting set volumes of the solution into Vacutainer tubes, evaporating the tubes to dryness under a gentle stream of nitrogen at 30°C and reconstituting the paracetamol in 1,0 ml of blank serum. The spiked serum samples were prepared for injection according to the method described in Section 4.2.5 and 5 to 15 microlitre of the clear supernatant liquid was injected onto the column. All of the determinations were done in triplicate and each sample was injected twice.

The paracetamol peak heights were expressed as a fraction of the internal standard peak heights, and the calibration curve was constructed by plotting the peak height ratios versus concentration. (The calibration curve is shown in Figure 4.6). The slope of the curve is 0,0534, the Y-intercept is -0,0041 and the correlation coefficient is 0,99959.

4.3.2 The Calibration Curves of Paracetamol and its Glucuronide and Cysteine Metabolites in Urine

The paracetamol, paracetamol glucuronide and paracetamol cysteine calibration curves were determined simultaneously. Urine samples were spiked by evaporating to dryness set aliquots of a methanolic solution of the compounds, as in Section 4.3.1, and reconstituting them in 1,0 ml of blank urine. The metabolites did not readily dissolve in the methanol and had to be placed in an ultrasonic bath for approximately 10 minutes to effect dissolution. The spiked urine samples were prepared for injection according to the method

described in Section 4.2.6 and 1 to 10 microlitre of the clear supernatant liquid was injected onto the column. All of the determinations were done in triplicate and each sample was injected twice.

The calibration curves were constructed by plotting peak height ratio (drug/internal standard) _versus_ concentration. Details of the calibration curves are given in Table 4.1 and the individual calibration curves are shown in Figures 4.7, 4.8 and 4.9.

TABLE 4.1 Details of the paracetamol, paracetamol glucuronide and paracetamol cysteine calibration curves in urine.

COMPOUND	CONCENTRATION RANGE, mcg/ml	SLOPE	Y-INTERCEPT	CORRELATION COEFFICIENT
Paracetamol	10 to 250	0,0010	0,0001	0,99934
Paracetamol glucuronide	25 to 5000	0,0007	0,0175	0,99979
Paracetamol cysteine	5 to 500	0,0008	-0,0017	0,99967

4.3.3 The Calibration Curve of Paracetamol Sulphate in Urine

The paracetamol sulphate calibration curve was not constructed in combination with the other drugs because the metabolite sample was found to be contaminated with paracetamol. For each successive determination of the calibration curve, the amount of paracetamol in the spiked urine samples increased and the amount of paracetamol sulphate decreased. Furthermore, for a given determination of the calibration curve, there was no correlation between the amount of paracetamol sulphate and the amount of paracetamol in the spiked urine samples. This indicated that the presence of paracetamol in the metabolite sample was not due to the utilization of inadequate purification measures during the preparation of the sample, as was originally thought, but decomposition of the sulphate conjugate itself.

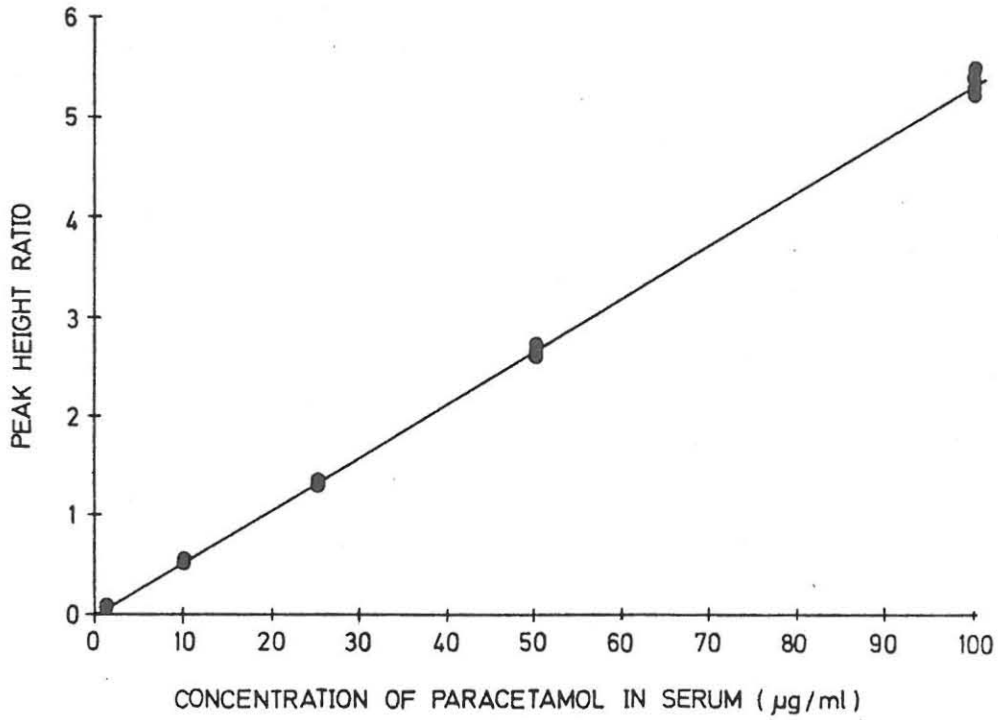


FIGURE 4.6 The calibration curve of paracetamol in serum

The curve is described by the expression

$y = 0{,}0534x - 0{,}0041 \quad (r = 0{,}99959)$

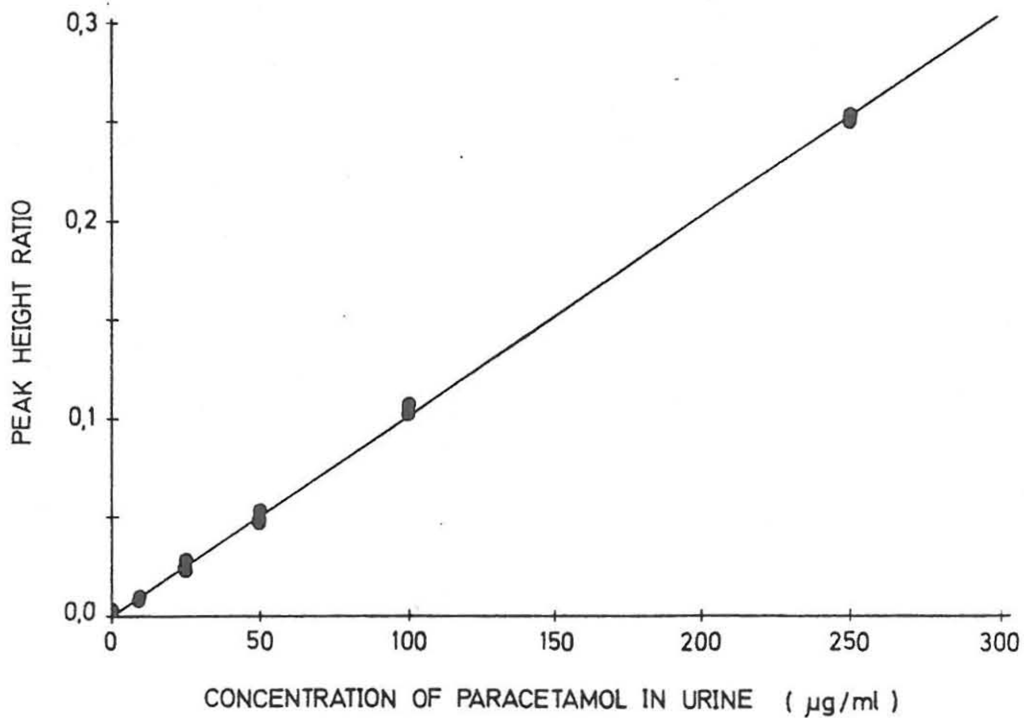


FIGURE 4.7 The calibration curve of paracetamol in urine

The curve is described by the expression

$y = 0{,}0010x + 0{,}0001 \quad (r = 0{,}99934)$

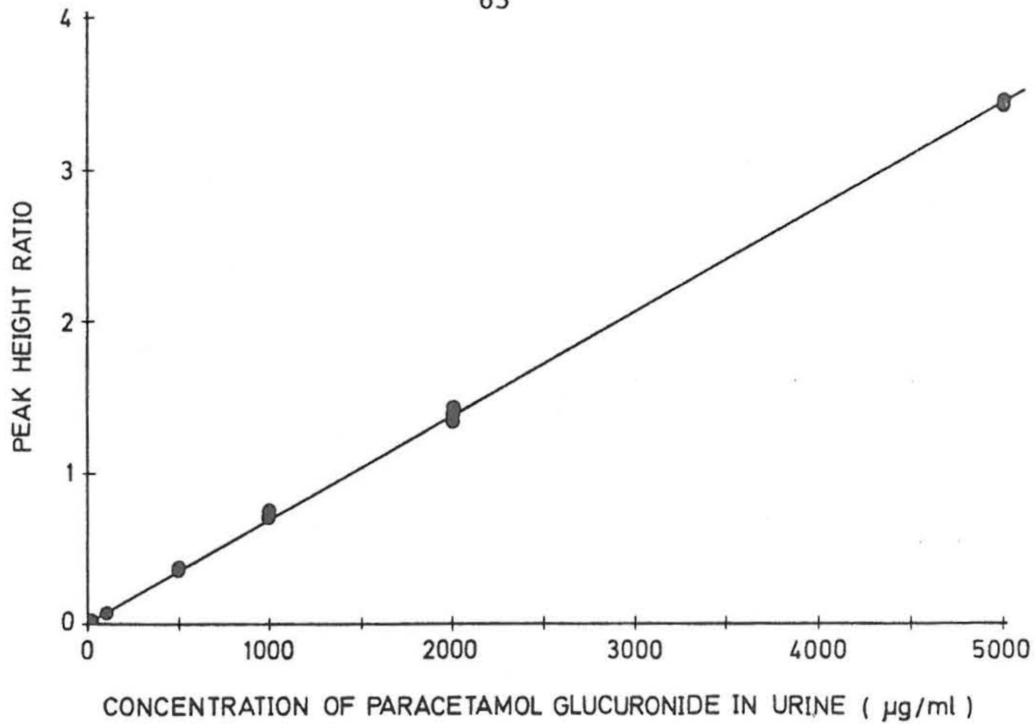


FIGURE 4.8 The calibration curve of paracetamol glucuronide in urine

The curve is described by the expression

y = 0,0007x + 0,0175 (r = 0,99979)

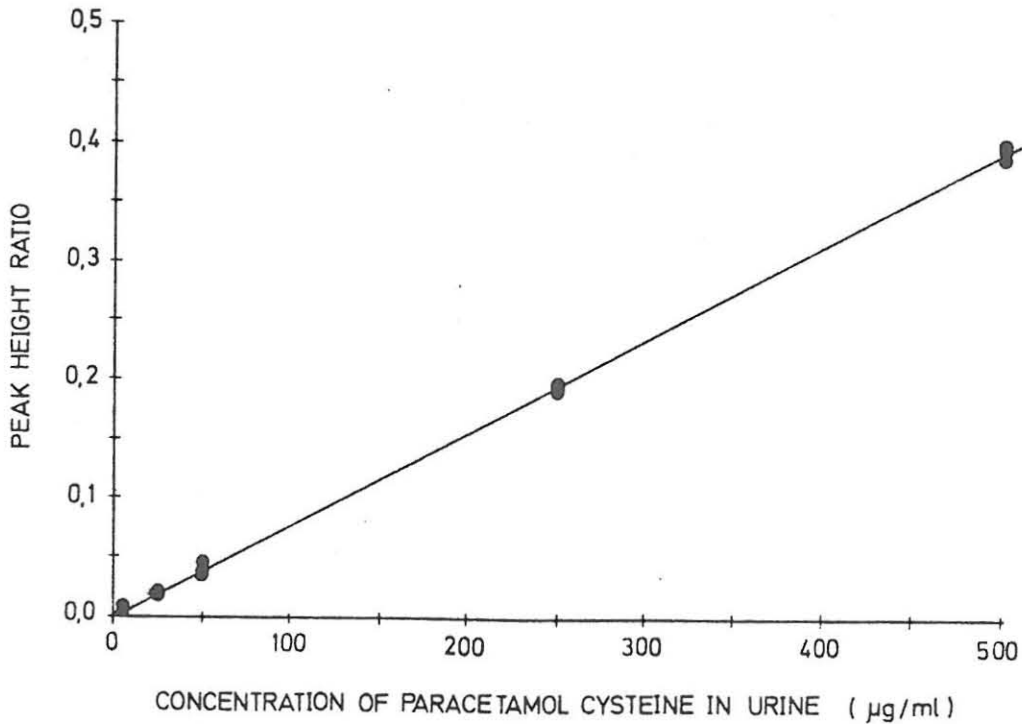


FIGURE 4.9 The calibration curve of paracetamol cysteine in urine

The curve is described by the expression

y = 0,0008x - 0,0017 (r = 0,99967)

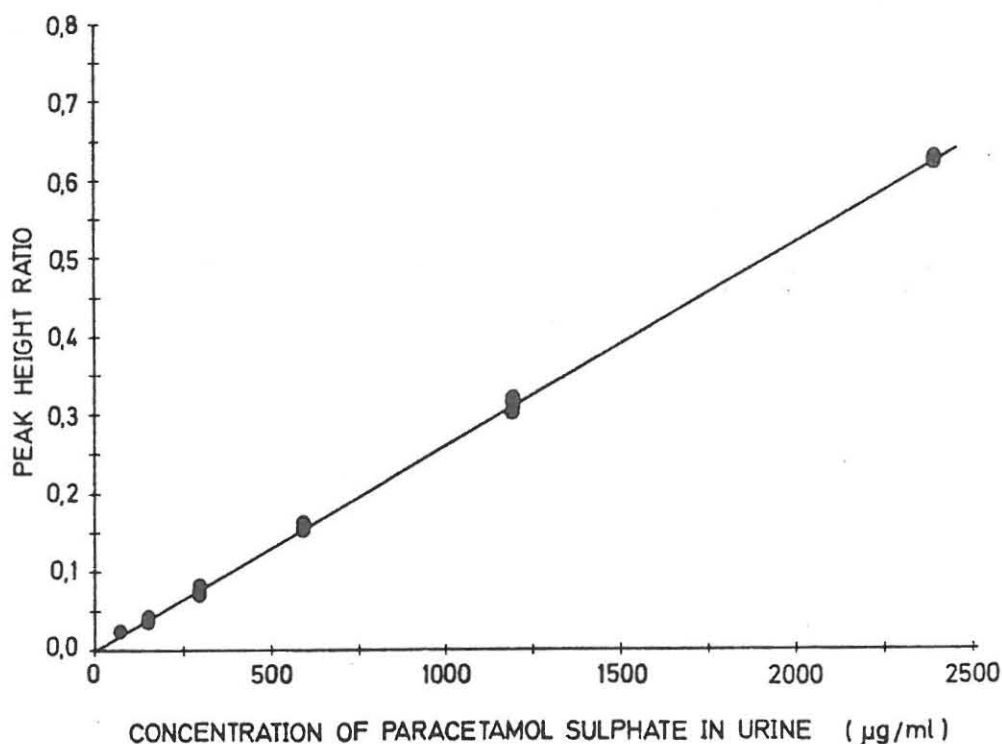


FIGURE 4.10 The calibration curve of paracetamol sulphate in urine

The curve is described by the expression

$y = 0,0003x + 0,0022$ $(r = 0,99968)$

The evaporation to dryness of the methanolic paracetamol sulphate solution in the Vacutainer tubes appeared to be the major cause of decomposition, as samples containing higher concentrations of the metabolite, which were subjected to longer periods in the drying block, were found to exhibit a markedly greater degree of decomposition than those samples containing lower concentrations of the metabolite. Storage of the spiked tubes in a dark cupboard prior to reconstitution in urine also appeared to contribute to the decomposition of the paracetamol sulphate, as progressively larger amounts of paracetamol were present in the samples for each replication of the calibration curve.

As a result of the above findings, a different method was employed for setting up dilutions for the paracetamol sulphate calibration curve. 5,0 mg of the metabolite was weighed out and dissolved in 1,0 ml of blank urine. The urine was prepared for injection according to the method described in Section 4.2.6 and serial dilutions of the resultant solution were made in the concentration range of 78 to

2500 mcg/ml. The original water-methanol-perchloric acid-urine ratio was maintained in all of the dilutions. Samples were centrifuged at 3000 rpm for 10 minutes and the clear supernatant liquid was injected onto the column. Insufficient paracetamol sulphate was available to enable the determinations to be done in triplicate. The calibration curve was therefore determined in duplicate and each sample was injected twice.

Under the new conditions, spiked urine samples still contained paracetamol. There was however a linear relationship between the amount of paracetamol and the amount of paracetamol sulphate in the samples, which was constant for both replications of the calibration curve. The concentration values were adjusted to eliminate the contribution made by paracetamol to these values and the calibration curve was constructed by plotting peak height ratios (paracetamol sulphate/internal standard) <u>versus</u> the adjusted concentrations. The calibration curve is shown in Figure 4.10. The slope of the curve is 0,0003, the Y-intercept is 0,0022 and the correlation coefficient is 0,99968.

4.4 RECOVERY AND PRECISION STUDIES

Recovery from serum was determined by spiking twelve Vacutainer tubes with 10 mcg of paracetamol and reconstituting six of these in 1,0 ml of blank serum and the rest in 1,0 ml of distilled water. All of the samples were prepared for injection according to the method described in Section 4.2.5 and 10 microlitre of the supernatant liquid was injected onto the column. The average concentration of the serum samples was expressed as a percentage of the average concentration of the aqueous samples and the percentage recovery was found to be 91,56%.

Recovery from urine was determined by spiking twelve Vacutainer tubes with 250 mcg of paracetamol and reconstituting six of these in 1,0 ml of blank urine and the rest in 1,0 ml of distilled water. The samples were prepared for injection according to the method described under Section 4.2.6 and 10 microlitre of the clear supernatant liquid was injected onto the column. The percentage recovery was calculated as for the serum samples and was found to be 100,75%. Ideally recovery studies should have been performed on the paracetamol

metabolites as well as the unchanged drug, but this was not possible as insufficient amounts of the metabolites were left over after the construction of the calibration curves.

The recovery study data were used to calculate "within run" precision as each recovery study was completed in a single run. The coefficient of variation was found to be 2,88% for the serum analysis and 1,15% for the urine analysis.

Standards containing 10 and 250 mcg of paracetamol were interspersed with the serum and urine samples obtained from the *in vivo* trial, respectively. The standards served to check that the system was functioning properly and also provided information on the day to day precision of the analysis. The coefficient of variation was found to be 2,19% for the serum analysis and 2,39% for the urine analysis.

CHAPTER 5

THE *IN VIVO* ASSESSMENT OF THREE BRANDS OF PARACETAMOL TABLETS

5.1 THE TRIAL PROTOCOL

5.1.1 Objective of the Trial

To ascertain whether or not significant differences observed between different brands of paracetamol tablets in an *in vitro* dissolution rate study are indicative of significant differences in bioavailability.

5.1.2 The Volunteers

Four apparently normal and healthy adults with no history of anaemia, renal disease or liver disease were used for the study. Details of the volunteers are given in Table 5.1.

TABLE 5.1 Details of the volunteers used in the trial

SUBJECT No.	CODE NAME	AGE (years)	BODY MASS (kg)	SEX
1	AK	29	59	male
2	DJ	33	75	male
3	DM	24	64	male
4	NA	20	51	female

The volunteers were non-smokers, moderate drinkers or abstainers and not allergic to analgesics derived from aniline. All of the volunteers received a typed copy of the protocol, and informed consent was obtained from each of them before admittance to the trial. Volunteers were paid an honorarium for participating in the trial.

5.1.3 The Treatment Plan

A 4 x 4 latin square design treatment plan was used for the trial. The sequence of administration is shown in Table 5.2. There was a "washout" period of one week between the first three doses. A nine

week interval elapsed before the last dose could be administered because some of the volunteers were affected by a month long holiday that started a day after the third trial and all of the volunteers were affected by a virulent influenza epidemic that occurred after the holiday. As a result of the unscheduled delays that were caused by the influenza epidemic, subject number 1 (AK) was unable to participate in the fourth trial.

TABLE 5.2 The treatment plan

	TRIAL NUMBER			
SUBJECT	1	2	3	4
NA	D	A	B	C
DM	C	D	A	B
DJ	B	C	D	A
AK	A	B	C	D

A = 1,0 g of paracetamol dissolved in 200 ml of water
B = 2 x 0,5 g Repamol tablets
C = 2 x 0,5 g Petersen paracetamol tablets (B/N 32449)
D = 2 x 0,5 g Propan paracetamol tablets

5.1.4 Standardization procedures

The volunteers had to be free from:

(i) all drugs, including "over the counter" preparations, for at least a week before the trial and for the duration of the trial. In the case of drugs that affect the liver enzymes, the restrictive period was one month.

(ii) tea, coffee, chocolate and cola drinks for at least 48 hours before the trial and for the duration of the trial.

(iii) all alcohol for at least 48 hours before the trial and for the duration of the trial.

(iv) all food and drink for 10 hours before the start of the trial and until the serving of the standard breakfast on the morning of the trial.

Volunteers were allowed to move about freely during the trial, but had to refrain from taking part in strenuous activities.

A standardized breakfast was served 2 hours after the start of the trial. It consisted of 250 ml of orange juice, 4 slices of toast, margarine and marmalade.

The administration of the test dose was staggered by 3 minute intervals, to enable blood samples to be collected from all of the volunteers at the correct time.

5.1.5 The Sampling Schedule for the Trial

	-20 min.	Blood drawn and urine collected for blanks
8-30 am	0 hr.	Test dose taken
8-45 am	15 min.	Blood drawn
9-00 am	30 min.	Blood drawn
9-15 am	45 min.	Blood drawn
9-30 am	1 hr.	Blood drawn
10-00 am	1,5 hr.	Blood drawn
10-30 am	2 hr.	Blood drawn and urine collected
	STANDARD	BREAKFAST
11.30 am	3 hr.	Blood drawn
12-30 pm	4 hr.	Blood drawn and urine collected
1-30 pm	5 hr.	Blood drawn
2-30 pm	6 hr.	Blood drawn and urine collected
	STANDARD	LUNCH
4-30 pm	8 hr.	Urine collected
8-30 pm	12 hr.	Urine collected
8-30 am	24 hr.	Urine collected

The tablets were swallowed intact with 200 ml of water. The control dose (1,0 g of paracetamol) was dissolved in 200 ml of water prior to ingestion.

5.1.6 <u>The Collection and Storage of the Blood Samples</u>

In three of the four volunteers a wide bore cannula (Medican 16GA, Medical Specialities, Randburg, Tvl, S.A.) was inserted into a large vein in the forearm. A 3-way stopcock (R62A Colour Course, Travenol Laboratories, Inc., Morton Grove, Illinois, U.S.A.) was attached to the cannula and was in turn connected to a reservoir of normal saline (Viaflex, Keagrams Ltd., Johannesburg, S.A.) by means of a fluid administration set (Plexitron, Keagrams Ltd., Johannesburg, S.A.). The normal saline contained 2 i.u./ml of heparin. The cannula, stopcock and lower end of the fluid administration set were securely strapped into position with adhesive tape, to enable the volunteers to retain mobility of the arm. (See Plate 5.1).

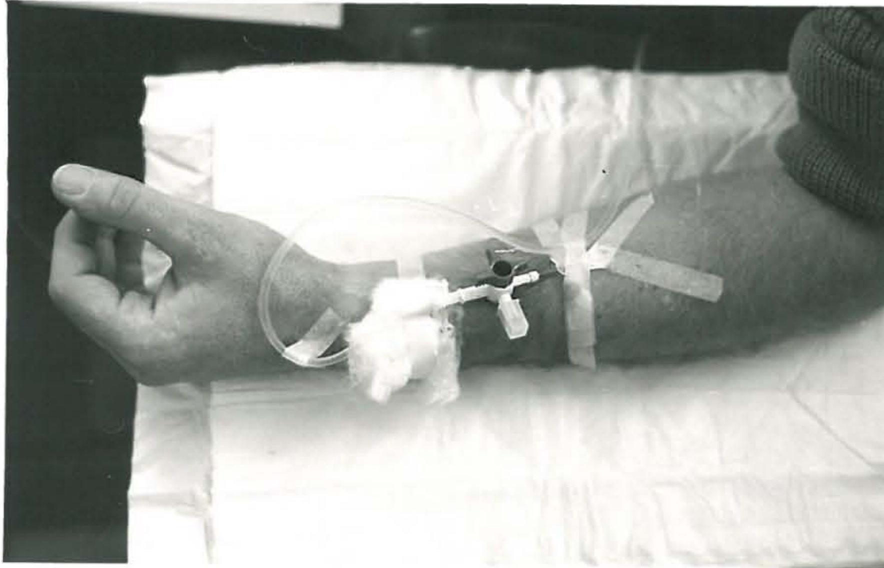


PLATE 5.1 The 3-way stopcock-indwelling cannula assembly utilized in the bioavailability trial.

In the periods between sampling the saline reservoir was linked to the cannula (stopcock position I in Figure 5.1) and saline was run into the vein at a flow rate of 8 ml/hour, the minimum rate that could be maintained, in order to keep the cannula clear. Immediately prior to the withdrawal of a blood sample the cannula was linked to the sampling port (stopcock position II in Figure 5.1), thus interrupting the flow of saline into the vein. The sampling path was cleared of saline by the withdrawal of about 2 ml of fluid and 10 ml of blood

was then collected for analysis by syringe aspiration. After sampling the system was flushed with about 2 ml of saline while the stopcock was in position I and III (Figure 5.1). This served to clear the cannula and sampling path of blood.

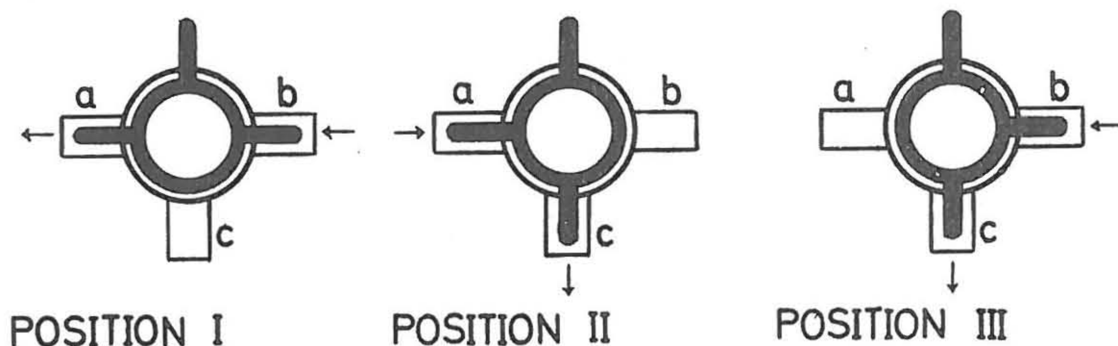


FIGURE 5.1 The control of fluid flow by different positions of the 3-way stopcock

(a) is connected to the cannula, (b) is connected to the saline reservoir and (c) is the sampling port.

Subject number 3 in Table 5.1 (DM) experienced discomfort when the cannula was positioned in the first trial and was therefore not willing to have samples collected in this way. As a result of this the cannula was removed and samples were obtained by means of multiple venopuncture in his case.

Blood samples were transferred from the syringe in which they had been collected to a labelled Vacutainer tube without delay. The tubes were stoppered and allowed to stand at room temperature for an hour to clot. Samples were then centrifuged at 3000 rpm for 10 minutes and the serum was transferred to a clean Vacutainer tube. The serum samples were stored at $-20^{\circ}C$ until analysis. Storage time did not exceed 5 days.

In a number of cases large fibrin clots were encountered in the serum. Entrapped serum was squeezed from the clot with the aid of an orange stick and the samples were respun to remove disturbed clot fragments before the serum was transferred to a clean tube.

A medical doctor was in attendance for the first 2 hours of the trial and remained on call for the remainder of the trial. A qualified theatre sister was in attendance until all of the blood samples had been collected and the cannulas had been removed from the forearms of the volunteers.

5.1.7 The Collection and Storage of the Urine Samples

The total urine output at each sampling time was collected in a large jar. Volunteers were asked to empty their bladders completely each time. The total volume of the urine was measured, a representative sample of 20 to 40 ml was transferred to a labelled, screw-cap bottle and the rest of the urine was discarded. Urine samples were frozen immediately after collection and stored at $-20^{o}C$ until analysis. Storage time did not exceed 3 weeks.

Urine samples passed at times other than those specified in the trial protocol, were collected in the same manner as above. In addition to the total urine volume the exact time at which the urine had been passed was also recorded in these cases.

After each collection the jars and measuring cylinders were thoroughly rinsed with water and placed upside down to drain, so as to be ready for the next collection. The importance of a rigorous adherence to the sampling procedure was stressed in both verbal and written instructions to the volunteers, since the collection of urine samples was largely left in the hands of the volunteers themselves and could therefore not be subject to the same degree of control as the collection of the blood samples.

5.2 The Analysis of the Serum Samples

Serum samples were brought to room temperature and mixed on a vortex mixer before being analysed in duplicate. The order in which they were analysed was randomized in order to avoid sequential effects. The samples were prepared for injection according to the method described in Section 4.2.5 (page 58) and 10 microlitre of the clear supernatant liquid was injected onto the column. The chromatographic conditions for the analysis are given below and a typical chromatogram is shown in Figure 5.2.

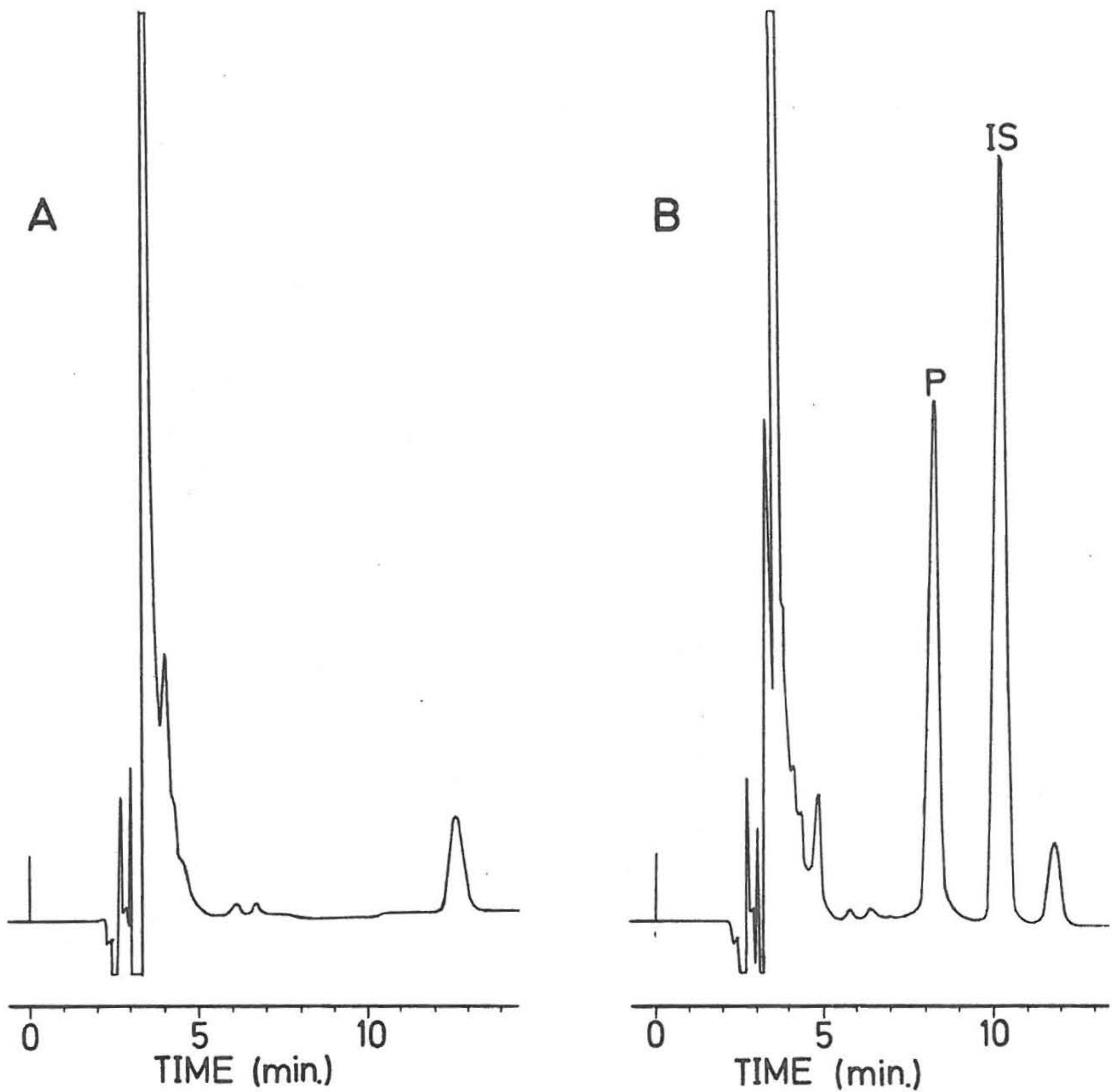


FIGURE 5.2 Chromatograms of (A) blank serum and (B) serum collected half an hour after the ingestion of a 1,0 g dose of paracetamol.

MOBILE PHASE: acetonitrile-water-formic acid (10: 90: 0,1 v/v). TEMP.: 22°C.
Peak P is paracetamol and peak IS is anhydrous theophylline, the internal standard.

Column: reverse phase μ Bondapak C_{18} (Waters Associates)
30 cm x 3,9 mm i.d.

Mobile phase: acetonitrile-water-formic acid (10: 90: 0,1 v/v)

Detector wavelength: 254 nm

Detector range: 0,02 aufs

Back off (coarse): 5

Flow rate: 1,0 ml/min.

Column pressure: 900 to 1000 psi

Column temperature: 22°C

Chart speed: 5 mm/min.

Pen range: 10 mV.

The peak height ratios (paracetamol/internal standard) were expressed as concentration readings by means of the parameters that were obtained from the calibration curve of paracetamol in serum. (Section 4.3.1, page 60.) The results of the serum analyses are listed in Table 5.3 and the serum profiles are shown in Figures 5.4 to 5.8.

5.3 The Analysis of the Urine Samples

Urine samples were brought to room temperature and mixed by hand before being analysed in duplicate. The samples were prepared for injection according to the method described in Section 4.2.6 (page 58) and 2 to 10 microlitre of the supernatant liquid was injected onto the column. The samples were analysed in a random order to avoid sequential effects. Chromatographic conditions for the urine analyses were essentially the same as those listed in Section 5.2. The detector range was however changed to 0,16 aufs.

Difficulties were encountered with the urine analyses as the characteristics of the paracetamol sulphate peak proved to be unstable. The paracetamol sulphate peak was originally sharp and symmetrical in the presence of perchloric acid (see Figure 4.5 B, page 59), but it subsequently began to exhibit a progressively greater degree of tailing and eventually became a relatively short and broad peak. In addition to the changes in the shape of the paracetamol sulphate peak, the retention time of the peak gradually increased and this resulted in the overlapping of the paracetamol and paracetamol sulphate peaks. (See Figure 5.3.)

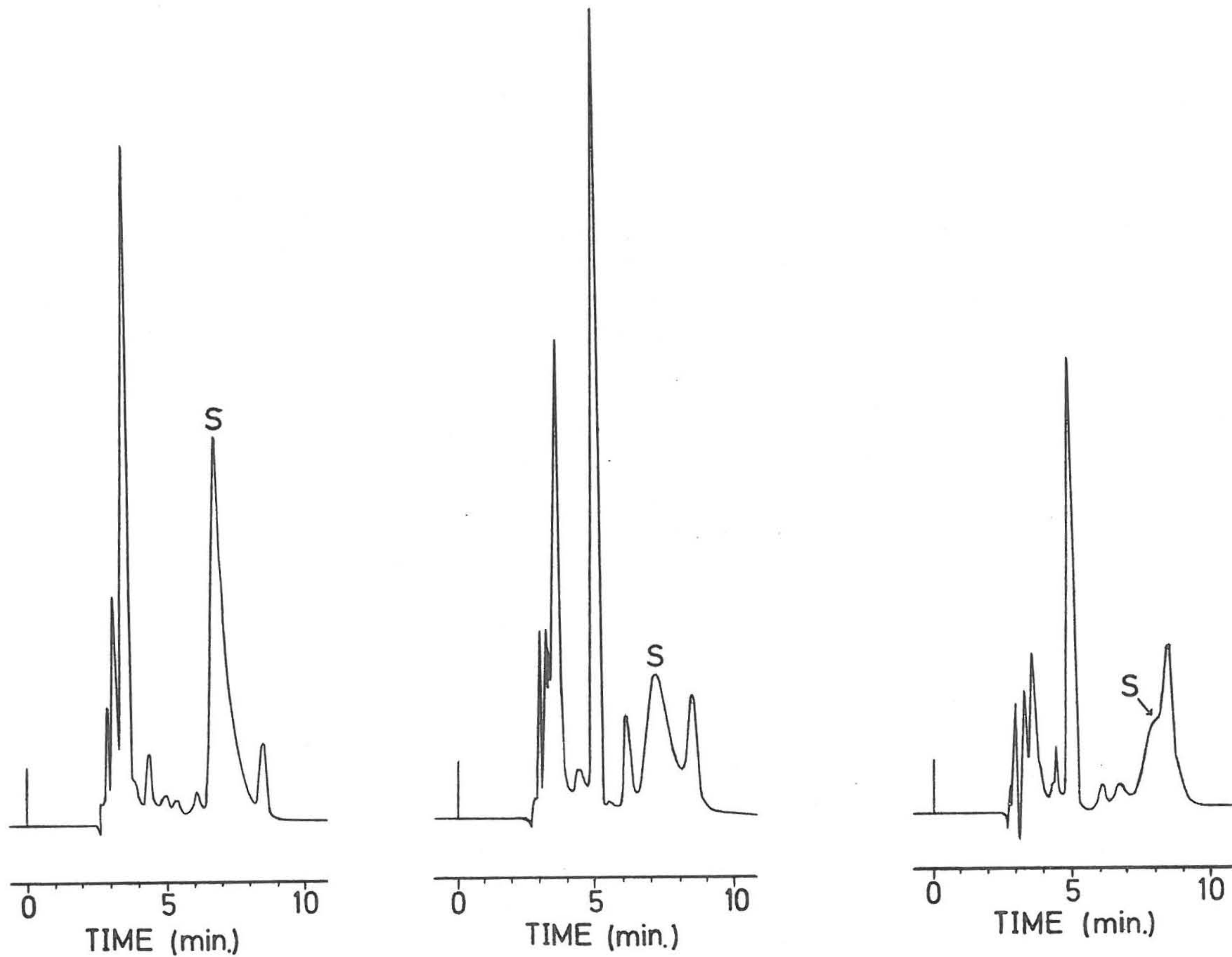


FIGURE 5.3 Chromatograms of urine samples illustrating the deterioration of the paracetamol sulphate peak (S). The chromatographic conditions are given in Section 5.3.

The reason for the unstable characteristics of the paracetamol sulphate peak could not be determined. Column deterioration did not appear to be a likely cause, as the retention times and shapes of the other peaks were unaffected and paracetamol standards continued to give expected values. For the same reasons, extracolumnar factors in the HPLC system were also ruled out as likely causes of the peak deterioration. A study was therefore undertaken to see whether the paracetamol sulphate itself had deteriorated on storage. Urine was collected 4 hours after the ingestion of 1,0 g of paracetamol and divided into four fractions. One fraction was frozen, another was refrigerated, a third was chromatographed shortly after collection and a fourth was left at room temperature for two days before being chromatographed. The chromatograms of the four urine fractions were almost identical. In addition to this, freshly prepared spiked aqueous samples containing perchloric acid yielded paracetamol sulphate peaks that were comparable to those in the urine. Sample deterioration therefore did not appear to be the cause of the changes in the characteristics of the paracetamol sulphate peak either.

Since insufficient samples of the paracetamol metabolites were available for the reconstruction of the calibration curves, far-reaching changes in the analytical method could not be considered. It was therefore decided to retain the results from the earlier analyses and to continue analysing the remainder of the urine samples by means of the existing method of analysis, in order to obtain as much information about the excretion of paracetamol as possible under the circumstances. Results from the urinary study were however viewed with reservations.

The amount of paracetamol, paracetamol glucuronide and paracetamol cysteine excreted in the urine at each time interval was determined and expressed as paracetamol equivalents. These values were used to calculate the cumulative percentage of the dose excreted. Results are listed in Table 5.4 and the average excretion profiles for the four test doses are presented in Figure 5.9.

5.4 RESULTS AND DISCUSSION

The scale of the *in vivo* bioavailability study was severely limited by the lack of suitable facilities for a large-scale trial in these laboratories and the fact that only one medical doctor was available

TABLE 5.3 Serum paracetamol levels after the oral administration of 1,0 g doses of the drug

A = 1,0 g paracetamol (BP)
B = 2 x 0,5 g Repamol tablets
C = 2 x 0,5 g Petersen paracetamol tablets (B/N 32449)
D = 2 x 0,5 g Propan paracetamol tablets

Each reading represents the average of two determinations. The standard deviation appears below each mean in parentheses.

TEST DOSE	SUBJECT	PARACETAMOL SERUM CONCENTRATION (mcg/ml) AT EACH SAMPLING TIME (hrs.)									
		0,25	0,5	0,75	1,0	1,5	2,0	3,0	4,0	5,0	6,0
A	AK	–	12,278	–	10,321	10,162	8,140	6,033	4,862	3,514	2,437
			(0,066)		(0,053)	(0,013)	(0,013)	(0,000)	(0,066)	(0,066)	(0,027)
	DJ	15,358	18,205	12,072	10,246	8,345	6,857	5,162	4,066	2,952	2,109
		(0,079)	(0,000)	(0,066)	(0,053)	(0,040)	(0,132)	(0,093)	(0,026)	(0,040)	(0,066)
	DM	11,707	13,177	13,458	12,438	10,293	8,374	5,977	4,394	2,999	2,456
		(0,000)	(0,013)	(0,040)	(0,053)	(0,093)	(0,080)	(0,026)	(0,013)	(0,000)	(0,053)
	NA	22,503	20,630	19,404	16,875	13,580	11,164	7,643	4,722	3,336	2,016
		(0,146)	(0,040)	(0,159)	(0,185)	(0,053)	(0,053)	(0,106)	(0,026)	(0,027)	(0,013)
B	AK	6,183	7,269	8,393	11,623	9,628	8,523	5,893	4,020	3,289	2,409
		(0,026)	(0,026)	(0,053)	(0,120)	(0,027)	(0,027)	(0,093)	(0,013)	(0,040)	(0,013)
	DJ	–	0,892	–	4,273	10,171	7,522	5,621	4,076	2,821	2,007
			(0,066)		(0,053)	(0,318)	(0,120)	(0,053)	(0,066)	(0,119)	(0,026)
	DM	3,467	7,495	11,004	11,669	11,051	10,031	6,491	4,516	3,055	2,587
		(0,026)	(0,079)	(0,066)	(0,026)	(0,000)	(0,093)	(0,040)	(0,079)	(0,026)	(0,026)
	NA	3,486	7,194	11,951	12,306	11,304	9,638	6,604	4,179	2,568	1,744
		(0,053)	(0,185)	(0,053)	(0,027)	(0,040)	(0,040)	(0,013)	(0,000)	(0,027)	(0,027)
C	AK	25,377	16,145	13,870	11,707	9,160	8,795	6,726	4,965	3,552	2,671
		(0,027)	(0,027)	(0,120)	(0,053)	(0,106)	(0,093)	(0,053)	(0,079)	(0,013)	(0,040)
	DJ	0,246	6,679	8,505	13,233	10,312	7,475	4,862	3,963	2,840	2,053
		(0,000)	(0,013)	(0,105)	(0,040)	(0,040)	(0,026)	(0,040)	(0,066)	(0,013)	(0,040)
	DM	–	1,576	–	5,443	8,936	12,428	7,690	5,686	3,654	2,653
			(0,080)		(0,013)	(0,132)	(0,146)	(0,358)	(0,013)	(0,066)	(0,013)
	NA	5,639	12,746	13,729	14,300	11,894	9,357	6,051	3,710	1,959	1,407
		(0,159)	(0,040)	(0,000)	(0,119)	(0,026)	(0,066)	(0,026)	(0,079)	(0,013)	(0,026)
D	AK	–	–	–	–	–	–	–	–	–	–
	DJ	0,302	12,578	12,540	12,278	8,505	6,876	4,666	3,289	2,447	2,016
		(0,106)	(0,093)	(0,040)	(0,119)	(0,053)	(0,026)	(0,026)	(0,013)	(0,013)	(0,040)
	DM	2,016	11,052	12,718	11,670	10,612	8,851	5,958	4,638	3,093	2,119
		(0,040)	(0,053)	(0,079)	(0,132)	(0,093)	(0,146)	(0,159)	(0,013)	(0,026)	(0,106)
	NA	–	12,868	–	13,561	9,984	9,301	5,976	2,793	1,782	1,520
			(0,026)		(0,132)	(0,053)	(0,120)	(0,106)	(0,106)	(0,053)	(0,053)

TABLE 5.4 The total amount of paracetamol, paracetamol glucuronide and paracetamol cysteine excreted in the urine after the ingestion of a 1,0 g dose of the drug

A = 1,0 g paracetamol
B = 2 x 0,5 g Repamol tablets
C = 2 x 0,5 g Petersen paracetamol tablets (B/N 32449)
D = 2 x 0,5 g Propan paracetamol tablets

TEST DOSE	SUBJECT	THE PERCENTAGE OF THE DOSE EXCRETED AT EACH TIME INTERVAL (hrs.)					
		2,0	4,0	6,0	8,0	12,0	24,0
A	AK	16,465	36,754	50,935	58,567	67,290	75,330
	DJ	14,762	34,325	48,142	56,501	68,967	80,612
	DM	11,773	27,206	39,812	47,272	56,022	65,825
	NA	17,468	34,683	52,487	60,414	68,240	75,648
B	AK	14,460	37,230	52,156	61,594	71,855	80,900
	DJ	8,929	29,670	43,726	53,284	63,670	73,728
	DM	8,170	28,588	39,194	47,607	55,594	64,490
	NA	20,495	49,840	66,925	74,518	81,384	87,414
C	AK	18,073	40,997	57,653	67,001	75,641	83,419
	DJ	13,206	34,064	49,067	58,713	72,642	83,393
	DM	5,346	20,812	31,546	39,307	49,824	61,050
	NA	19,713	48,634	60,124	69,972	77,989	84,843
D	AK	-	-	-	-	-	-
	DJ	14,376	37,710	52,735	63,103	75,956	85,394
	DM	9,826	26,181	28,770	36,902	50,409	62,593
	NA	18,034	43,814	58,736	66,019	72,986	77,730

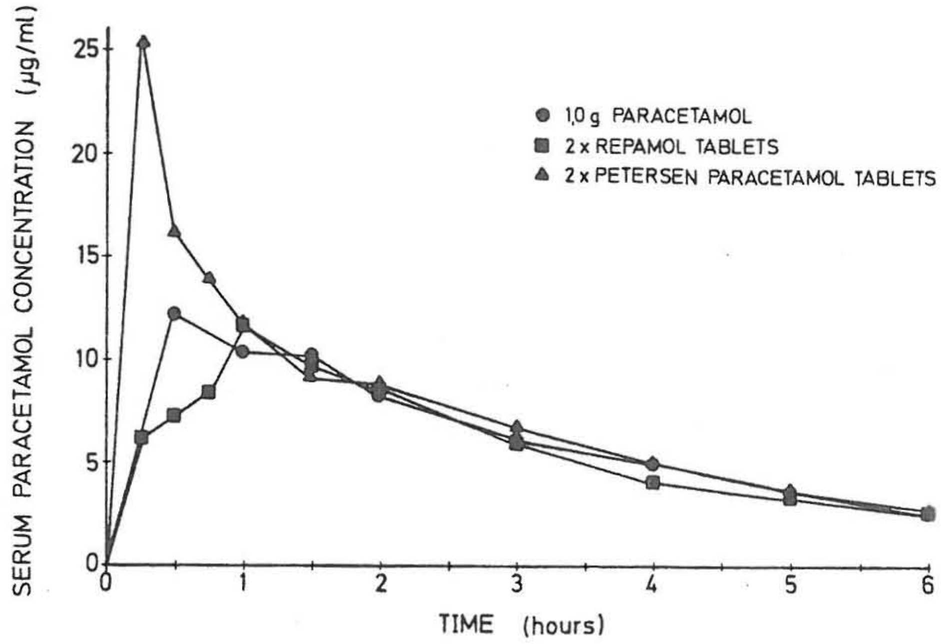


FIGURE 5.4 The serum levels obtained after the ingestion of three paracetamol test doses by subject AK.

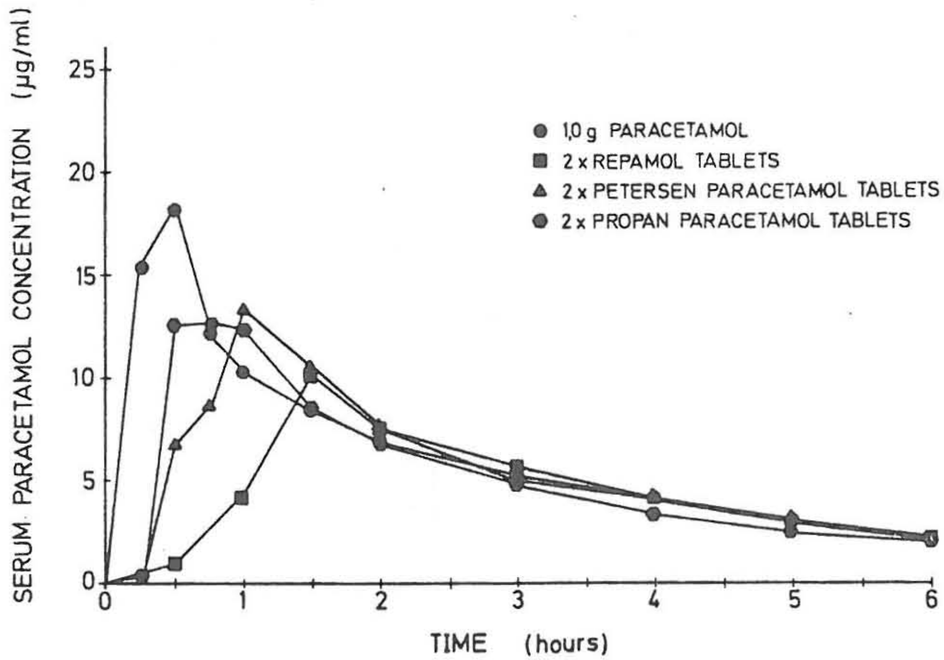


FIGURE 5.5 The serum levels obtained after the ingestion of four paracetamol test doses by subject DJ.

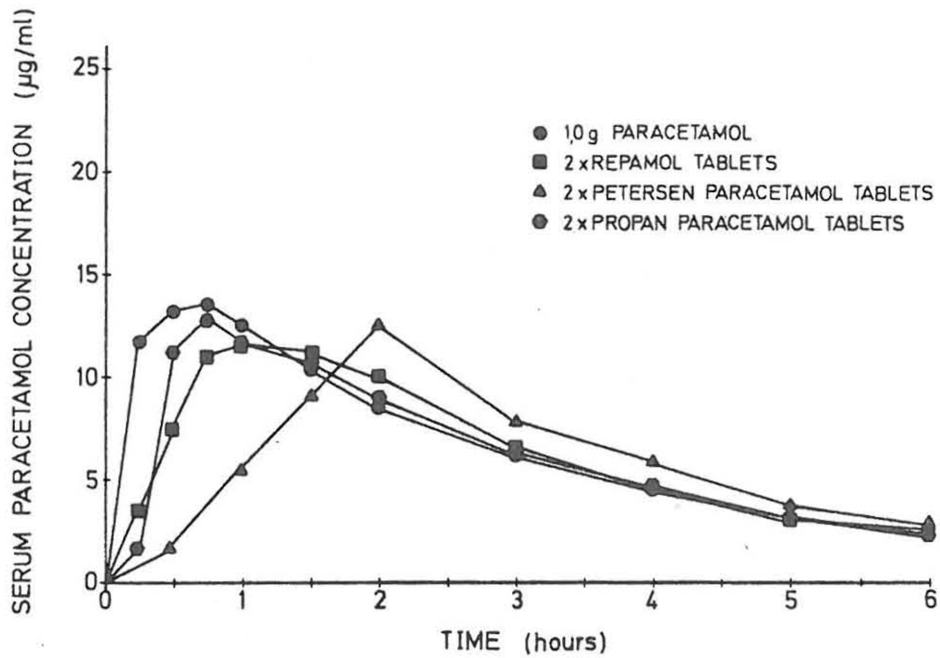


FIGURE 5.6 The serum levels obtained after the ingestion of four paracetamol test doses by subject DM.

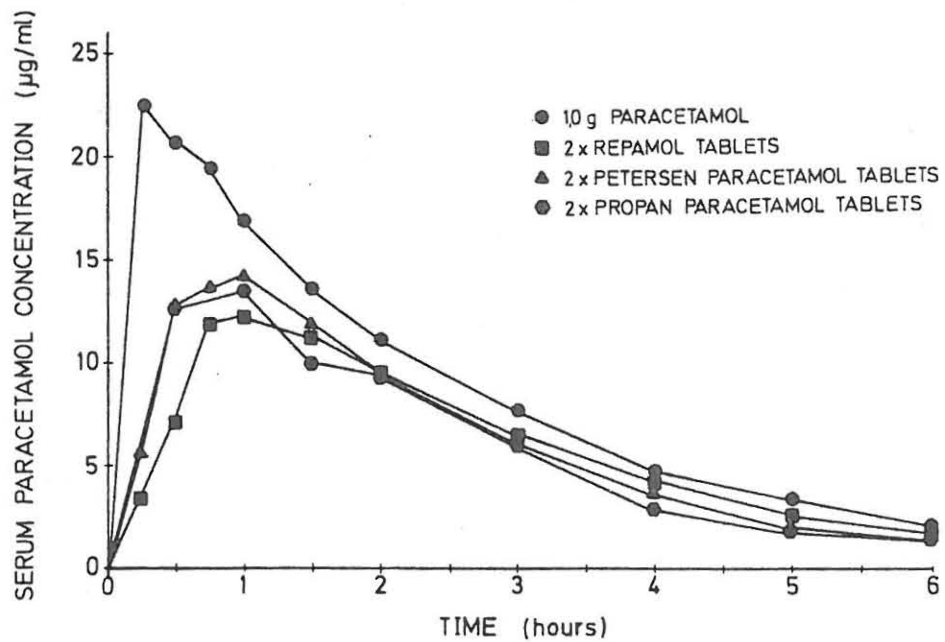


FIGURE 5.7 The serum levels obtained after the ingestion of four paracetamol test doses by subject NA.

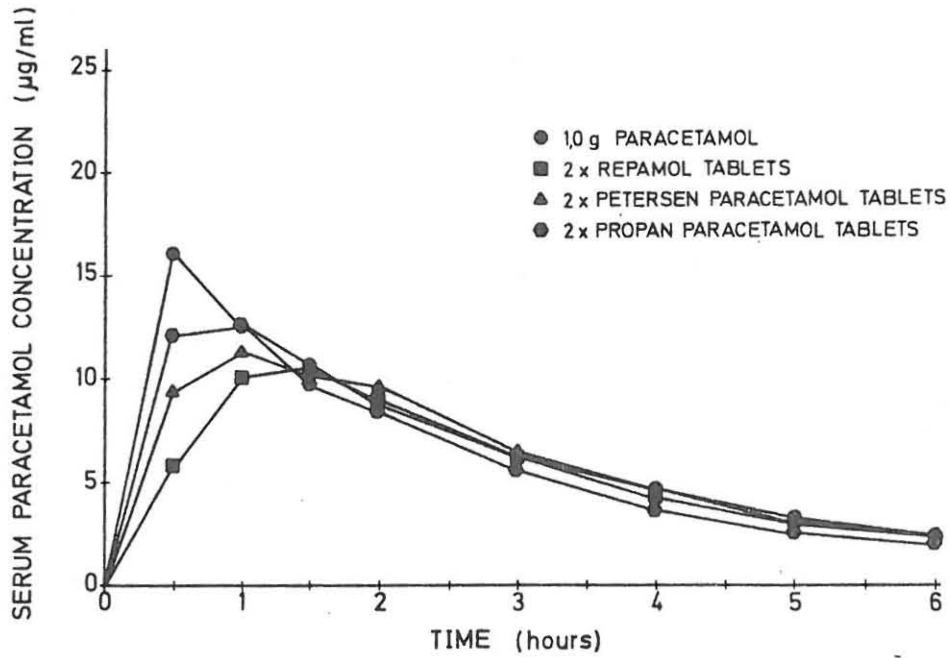


FIGURE 5.8 The average serum profiles of the four test doses of paracetamol.

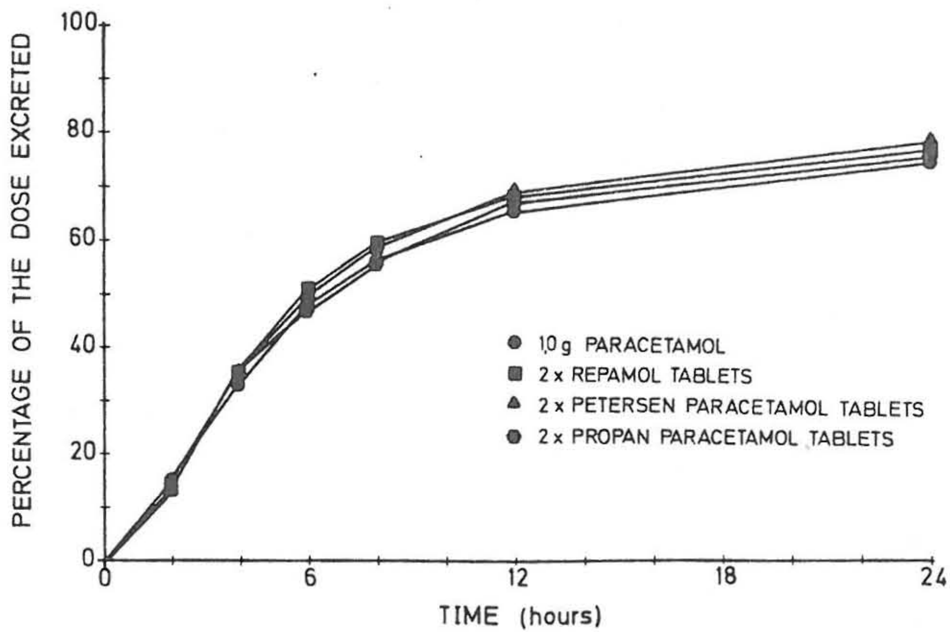


FIGURE 5.9 The average urinary excretion profiles of the four test doses of paracetamol.

to conduct the trial. Difficulties were also encountered in finding suitable volunteers for the trial. As a result of this, the number of volunteers was restricted to four and it was therefore not possible to investigate the bioavailability of all of the tablets that had been tested in the dissolution rate studies. The tablet with the fastest dissolution rate (Petersen paracetamol, B/N 32449), the tablet with the slowest dissolution rate (Repamol) and a tablet with an intermediate dissolution rate (Propan paracetamol) were selected for *in vivo* investigation in order to give as true a representation of the dissolution rate data as possible in the bioavailability study. A control dose of paracetamol dissolved in water was included in the trial for comparative purposes.

The use of an indwelling cannula and 3-way stopcock assembly for collecting blood samples was found to be convenient and resulted in less trauma at the injection site than multiple venopuncture. The stopcocks used initially were not very robust and a number of them had to be replaced during the trial because of breakages. This problem was not encountered when another batch of 3-way stopcocks was used.

Quarter and three quarter hour blood samples were not collected in the first trial. This omission was due to delays in the sampling schedule that were caused by the removal of the cannula from volunteer DM, who had requested to change over to multiple venopuncture, and problems that were encountered with faulty stopcocks. Apart from the above omission and the fact that subject AK was unable to participate in the fourth trial (see Section 5.1.3), the *in vivo* study followed the prescribed protocol closely.

The method of analysis adopted for the study proved to be rapid and reproducible with the exception of the paracetamol sulphate peak. More than 1000 samples were injected onto the column without any adverse effects on the column pressure or the retention times of paracetamol, paracetamol glucuronide, paracetamol cysteine and theophylline, the internal standard.

Peak blood levels generally occurred 30 to 90 minutes after the ingestion of the dose and peak blood levels varied from 10 to 25 mcg/ml. These values agree well with the literature. About 76% of the dose

could be accounted for in the urine in the form of free drug and its glucuronide and cysteine metabolites. Approximately 63% of the dose was excreted as the glucuronide, 5% as the cysteine and 7% as the unchanged drug. The latter two values are higher than those that are generally quoted in the literature. (See Section 1.2.5, page 5.)

CHAPTER 6

METHODS OF EXPRESSING IN VIVO BIOAVAILABILITY DATA

6.1 INTRODUCTION

Single-dose blood level curves are generally characterized by the peak concentration (c_{max}), the time of the peak concentration (t_{max}) and the area under the blood level curve (AUC).[182] The first two parameters are associated with the rate of absorption and the third parameter is associated with the extent of absorption.

The peak time and the peak concentration can be obtained directly from the blood level curve. The AUC can be calculated by means of the trapezoidal rule[183] which is summarized by Equation 6.1.

$$AUC_{0\rightarrow t_x} = \left(\frac{c_1 \cdot t_1}{2}\right) + \left(\frac{c_1+c_2}{2} \cdot (t_2-t_1)\right) + \left(\frac{c_2+c_3}{2} \cdot (t_3-t_2)\right) + \ldots\ldots\ldots + \left(\frac{c_{(x-1)}+c_x}{2} \cdot (t_x-t_{(x-1)})\right) \quad (6.1)$$

where 'c_1' is the concentration at 't_1', 'c_2' is the concentration at 't_2', etc. The AUC can be extrapolated to infinity[183] according to Equation 6.2.

$$AUC_{0\rightarrow\infty} = AUC_{0\rightarrow t_x} + \frac{c_x}{k} \quad (6.2)$$

where 'c_x' is the concentration at the last sampling time and 'k' is the overall elimination rate constant of the drug.

The AUC reflects not only the extent to which a compound has been absorbed, but also the elimination rate of the compound. The pharmacokinetically more valid product of the AUC and the elimination rate constant is therefore sometimes used in place of the AUC to assess biological differences between formulations.[184]

Urinary excretion data are generally characterized by the cumulative amount of drug excreted at various time intervals.[7,16,21,30] The amount

of drug excreted in 24 hours (E_{24}) is the most commonly used parameter in comparative bioavailability studies.

The bioavailability parameters from the results of the in vivo study are listed in Table 6.1. The quarter and three quarter hour readings from the blood level data were ignored when the parameters were derived, as these readings were not obtained in the first trial.

6.2 THE STATISTICAL ANALYSIS OF RESULTS

Results were evaluated by means of analysis of variance (ANOVA). The ANOVA was modified to take into account the fact that subject AK did not participate in the fourth trial (See Appendix 3) and the 95% confidence level was employed for the statistical analysis. Results of the analysis are listed in Table A.3. (See Appendix 4.)

6.3 RESULTS AND DISCUSSION

6.3.1 The Blood Level Data

There were no significant differences between the test doses for any of the bioavailability parameters tested. There were however highly significant subject differences for the elimination rate constant and the product of the $AUC_{0\rightarrow\infty}$ and the elimination rate constant. The subject differences were due to subject NA, who different significantly from all of the other volunteers.

6.3.2 The Urinary Excretion Data

Highly significant subject differences and significant trial number differences were found for the E_{24}. The subject differences were due to subject DM, who differed significantly from all of the other volunteers. In the case of the trial number, the first trial was found to differ significantly from the third trial. There were no significant differences between the treatments.

Analysis of the cumulative fraction of the dose excreted after 2, 4, 6, 8 and 12 hours indicated that there were no significant differences between treatments at the earlier readings either. As in the case of the E_{24}, highly significant subject differences were found at all of the sampling times and significant differences were found between the trials at the 6, 8 and 12 hour readings. In each case, the third trial

TABLE 6.1 Bioavailability parameters of the four paracetamol test doses.

A = 1,0 g Paracetamol

B = 2 x 0,5 g Repamol Tablets

C = 2 x 0,5 g Petersen Paracetamol Tablets (B/N 32449)

D = 2 x 0,5 g Propan Paracetamol Tablets

TEST DOSE	SUBJECT	t_{max} (hrs)	c_{max} (μg/ml)	$AUC_{0\rightarrow 6}$ ((μg/ml).hr)	$AUC_{0\rightarrow\infty}$ ((μg/ml).hr)	k (hr^{-1})	CORR. COEFF. for k	$AUC_{0\rightarrow\infty}.k$ (μg/ml)	E_{24} (% of dose)
A	AK	0,5	12,278	38,113	46,397	0,304	0,9937	14,105	75,330
	DJ	0,5	18,205	36,775	43,942	0,301	0,9971	13,227	80,612
	DM	0,5	13,177	38,832	46,570	0,305	0,9934	14,204	65,825
	NA	0,5	20,630	50,624	55,358	0,434	0,9977	24,025	75,648
B	AK	1,0	11,623	35,059	43,384	0,288	0,9934	12,495	80,900
	DJ	1,5	10,171	26,831	32,642	0,346	0,9997	11,294	73,728
	DM	1,0	11,669	37,985	45,705	0,315	0,9873	14,397	64,490
	NA	1,0	12,306	36,854	40,652	0,448	0,9990	18,212	87,414
C	AK	0,5	16,145	41,681	50,207	0,310	0,9996	15,564	83,419
	DJ	1,0	13,233	33,410	40,606	0,292	0,9947	11,857	83,393
	DM	2,0	12,428	35,646	42,870	0,364	0,9972	15,605	61,050
	NA	1,0	14,300	38,911	41,547	0,501	0,9934	20,815	84,843
D	AK	-	-	-	-	-	-	-	-
	DJ	0,5	12,578	33,248	40,123	0,281	0,9923	11,275	85,394
	DM	1,0	11,670	38,054	44,236	0,351	0,9954	15,527	62,593
	NA	1,0	13,561	36,493	39,366	0,456	0,9591	17,951	77,730

differed significantly from the first and second trials. Subject DM differed significantly from all of the other volunteers at all of the sampling times and subjects AK and NA were significantly different at the 6 hour reading. DJ differed significantly from NA up to the 8 hour reading and differed from AK at the 2 hour reading.

6.3.3 General Discussion

The rank order of the treatment totals derived during the statistical analysis of the bioavailability parameters was investigated and the order was found to be A > C > B > D for the c_{max}, $AUC_{0 \rightarrow 6}$, $AUC_{0 \rightarrow \infty}$, $AUC_{0 \rightarrow \infty}.k$ and the 2 hour urine sample; B > C > A > D for the 4 to 8 hour urine samples; and C > B > A > D for the 12 and 24 hour urine samples as well as the elimination rate constant. The rank order of the t_{max} was B=C > D > A. None of these rank orders correspond to the order of the dissolution rates, which is A > C > D > B. The <u>in vitro</u> rank order of the dosage forms was based on the rank order of the $t_{50\%}$, the scale parameter (a) and the dissolution time (Td), since these parameters would appear to be the most pertinent indicators of the dissolution rates of the dosage forms. The location parameter (Ti), by virtue of its definition, could also be expected to give some information on the dissolution characteristics of the dosage forms. The parameter however did not establish a definite trend as the $t_{50\%}$, the scale parameter and the dissolution time had done, and it was therefore not taken into account. The rank order of the location parameter was A < B < C < D in the manual USP rotating basket assembly, A < D < B < C in the automated USP rotating basket assembly and A < B < D < C in the stationary basket-rotating paddle apparatus. In all of the above cases, the control dose (A) was assumed to have the highest dissolution rate (<u>i.e.</u> the smallest $t_{50\%}$, a, Td and Ti values) since paracetamol was in the form of an aqueous solution in this test dose.

As a result of the fact that the quarter and three quarter hour readings from the blood level data had to be ignored when the bioavailability parameters were derived, the precision with which these parameters describe the serum profiles was decreased. This fact is illustrated by subjects DM, DJ and NA who were found to have a t_{max} value of 0,5 hours (see Table 6.1) after the ingestion of test dose A. Inspection of the raw serum level data in Table 5.3 however shows that NA in fact has a t_{max} of 0,25 hours and DM a t_{max} of 0,75 hours. The t_{max} quoted for DJ remains unchanged.

The statistical evaluation of the blood level data indicated that no significant differences could be attributed to subject DM. This would tend to confirm that the two methods of withdrawing blood samples that were employed in this study (*i.e.* multiple venopuncture and an indwelling cannula-3-way stopcock assembly) had no significant effect on the serum profiles that were obtained from the trial.

The elimination half life of paracetamol ranged from 1,51 ± 0,09 to 2,31 ± 0,09 hours in this study. The average half life was found to be 2,03 ± 0,36 hours. These values agree well with the literature. (See Section 1.2.4, page 4.)

CHAPTER 7

CONCLUSIONS

The statistical assessment of the parameters obtained from the dissolution rate data indicated that Repamol, which consistently dissolved more slowly than the other tablets, and Petersen paracetamol (B/N 32449), which consistently dissolved more rapidly than the majority of the other tablets, differed significantly from one another when all of the dissolution parameters were investigated with the exception of the shape parameter (b) and the location parameter (Ti). Propan paracetamol had an intermediate dissolution rate and yielded a dissolution profile that exhibited characteristics which differed significantly from Repamol in certain instances and charactetistics which differed significantly from Petersen paracetamol in other instances. (See Appendix 1 and 2.)

On the basis of the *in vitro* results there is therefore evidence that real differences exist between the tablets that were subsequently tested in the *in vivo* trial. The inter-tablet differences would appear to be consistent as the dissolution characteristics of Repamol, Propan paracetamol and Petersen paracetamol (B/N 32449) were the same overall in both the USP rotating basket assembly and the stationary basket-rotating paddle apparatus.

In the case of the *in vivo* results, no significant differences could be demonstrated between the different test doses and no direct parallels could be drawn between the ranking of the bioavailability parameters and the dissolution parameters ($t_{50\%}$, a and Td). The ranking of the treatment totals for the c_{max}, $AUC_{0\rightarrow 6}$, $AUC_{0\rightarrow\infty}$, $AUC_{0\rightarrow\infty}.k$ and the E_2 ($A>C>B>D$) comes the closest to the rank order of the dissolution parameters ($A>C>D>B$).

The results of this study would tend to indicate that the differences observed between the dissolution profiles of the given paracetamol tablets, though highly significant at the 95% confidence level in many cases, were not sufficiently large to influence the bioavailability of the tablets to a significant extent and that some factor or combination of factors other than dissolution rate, played a rate-limiting role in the absorption of paracetamol. The fact that there

were not only no significant differences between the tablets tested in the *in vivo* study, but no significant differences between these tablets and the control dose of 1,0 g of paracetamol dissolved in water, would tend to confirm the hypothesis that the dissolution rate of the dosage forms had a minimal effect on the absorption rate of paracetamol.

A number of factors have been reported to influence the absorption rate of paracetamol. (See Section 1.2.1, page 2.) The presence of food in the gastrointestinal tract is not likely to have been the cause of anomalous results in this study as all of the test doses were taken after an overnight fast, food was withheld for two hours after the ingestion of the test doses and food intake was strictly controlled for the first six hours of the trial.

Age differences between the volunteers may have influenced the results of the trial. The volunteers who participated in the trial were between 20 and 33 years old, with an average age of 26,5 years. It has been reported that the best absorption occurs in the 30 to 40 year old age group. Subject DJ falls into this age group and was found to differ significantly from subjects DM (24 years) and NA (20 years) when the 2, 4, 6 and 8 hour urinary excretion values were investigated and from subject DM alone when the 12 and 24 hour urinary excretion values were investigated. Inspection of the relevant data showed that the subject totals derived for DJ during the statistical assessment of the urinary excretion data, though larger than those for DM, were smaller than those for NA at any given sampling time. The subject differences ascribed to subject DJ could therefore not be assumed to indicate improved absorption of the drug. In view of this finding and the relatively small age differences that existed between the volunteers, age would not appear to have had far-reaching effects on the results of this study.

The rate of gastric emptying, which has been shown to correlate well with the rate at which paracetamol is absorbed, is likely to be the most probable single factor of those mentioned in Section 1.2.1 to play a rate-limiting role in the absorption of paracetamol. Gastric emptying patterns have been found to show considerable variation,[185] even in trials where the conditions have been carefully controlled. Variations in the gastric emptying pattern from trial to trial may

therefore account for the anomaly that was noted in the serum profiles of subject AK. In this case a formulated product gave rise to a peak serum concentration that was approximately twice as high as that observed after the ingestion of the control dose of paracetamol in solution. (See Figure 5.4, page 79.) For all of the other volunteers the control dose gave rise to a serum profile that was comparable to or higher than those of the formulated products.

The subject differences that were found between subject DM and the other volunteers when the urinary excretion data was investigated may have been related to the fact that blood samples were obtained from this volunteer by means of multiple venopuncture. The differences are not likely to have been caused directly by the method of sample collection, but may have resulted from the greater freedom of movement that DM enjoyed. The volunteers who were fitted with an indwelling cannula remained seated virtually throughout the first six hours of the trial because they were encumbered by the saline drip. Subject DM was able to walk about freely in the building. This discrepancy in posture between the two sets of volunteers may have influenced the absorption pattern of the drug, since body position has been related to the rate at which paracetamol is absorbed. The additional fluid (8 ml/hour) that was taken in by the volunteers on the drip is not likely to have had a significant effect on the results. Apart from the fact that urinary flow rates have a limited effect on the renal clearance of unchanged paracetamol and no effect on the clearance of the metabolites of paracetamol, subject DM had an average urinary flow rate that was only 2 to 4 ml/hour lower than the other volunteers. The average urinary flow rate varied from 56 ml/hour (DM) to 60 ml/hour (AK).

There could be a number of reasons for the significant subject differences that were attributed to subject NA when the elimination rate constant and the product of the elimination rate constant and the extrapolated area under the serum level curve were assessed. The subject differences may have been due to sex, age or weight since subject NA was the only female in the trial, she was the youngest of the volunteers and she weighed the least. Since biotransformation is the major route by which paracetamol is eliminated, the subject differences may however simply reflect an inherent, inter-individual variation in metabolic rate.

Apart from the above physiological effects, the lack of correlation between the results of the dissolution rate study and the bioavailability trial may have been due to the fact that only a small number of volunteers could be accommodated in the trial. The limited size of the bioavailability study could have resulted in the trial being too insensitive to detect real differences between the test doses. Inter-tablet differences may also have been suppressed by the fact that the results of the quarter and three quarter hour serum analyses could not be used for the derivation of the bioavailability parameters, and the fact that subject AK was unable to take part in the fourth trial.

The supposition that the differences in dissolution rate are too small to affect the bioavailability of the tablets significantly and that some factors other than dissolution rate played a more significant role in the absorption of paracetamol is probably the most likely explanation of the observed results. The effect of the small trial size and the deviations from the prescribed protocol can however not be discounted.

APPENDIX I

TABLE A.1 Results from the Scheffè test - USP rotating basket apparatus

* indicates significant differences at the 95% confidence level.

COMPARISON PAIRS		DIFFERENCE BETWEEN THE MEAN FRACTION DISSOLVED AT EACH TIME INTERVAL (min.)																		DISSOLUTION PARAMETERS				
		1,0	2,0	3,0	4,0	5,0	6,0	7,0	8,0	9,0	10,0	11,0	12,0	13,0	14,0	15,0	16,0	17,0	18,0	$t_{50\%}$	a	b	Ti	Td
Repamol	Propan	0,04	0,04	0,04	0,02	0,02	0,08	0,21	0,28	0,30*	0,32*	0,32*	0,32*	0,31*	0,29	0,28	0,26	0,24	0,22	4,13	9,41	0,42*	0,03	3,28
	Pet.(32978)	0,10	0,44*	0,60*	0,63*	0,62*	0,60*	0,57*	0,54*	0,51*	0,48*	0,46*	0,43*	0,40*	0,37*	0,34*	0,31*	0,28*	0,25	8,66*	10,20*	0,17	0,08	8,89*
	Pet.(32449)	0,14*	0,51*	0,63*	0,65*	0,65*	0,62*	0,58*	0,55*	0,52*	0,49*	0,46*	0,43*	0,40*	0,37*	0,34*	0,31*	0,28*	0,25	8,91*	10,79*	0,15	0,08	9,23*
	Panado	0,02	0,07	0,10	0,11	0,12	0,12	0,12	0,11	0,11	0,11	0,11	0,10	0,09	0,09	0,08	0,07	0,06	0,06	2,45	3,30	0,02	0,09	2,14
	Norstan	0,12*	0,26*	0,39*	0,47*	0,51*	0,52*	0,51*	0,49*	0,47*	0,45*	0,43*	0,41*	0,38*	0,35*	0,32*	0,30*	0,27	0,25	8,06*	9,70*	0,01	0,10	7,99*
	Napamol	0,03	0,03	0,05	0,05	0,06	0,08	0,08	0,09	0,10	0,12	0,13	0,13	0,14	0,13	0,13	0,13	0,12	0,11	2,26	2,63	0,00	0,03	1,91
	Dolorol	0,05	0,27*	0,49*	0,56*	0,58*	0,57*	0,55*	0,52*	0,50*	0,47*	0,45*	0,42*	0,39*	0,36*	0,33*	0,31*	0,28*	0,25	8,25*	8,97	0,18	0,07	8,16*
Propan Paracetamol	Pet.(32978)	0,14*	0,48*	0,63*	0,65*	0,61*	0,52*	0,36*	0,26	0,21	0,17	0,14	0,11	0,09	0,08	0,06	0,05	0,04	0,03	4,53	19,61*	0,25*	0,11	5,61
	Pet.(32449)	0,18*	0,55*	0,67*	0,68*	0,63*	0,54*	0,38*	0,27	0,22	0,17	0,14	0,12	0,09	0,08	0,06	0,05	0,04	0,03	4,78	20,20*	0,27*	0,11	5,95
	Panado	0,05	0,11	0,14	0,14	0,11	0,04	0,09	0,17	0,19	0,21	0,21	0,22	0,21	0,20	0,20	0,19	0,18	0,17	1,68	12,71*	0,44*	0,12	1,14
	Norstan	0,15*	0,31*	0,43*	0,49*	0,50*	0,44*	0,30*	0,22	0,17	0,14	0,11	0,09	0,07	0,06	0,04	0,04	0,03	0,02	3,93	19,11*	0,42*	0,12	4,71
	Napamol	0,06	0,08	0,08	0,08	0,05	0,00	0,13	0,19	0,20	0,20	0,19	0,18	0,17	0,16	0,15	0,14	0,12	0,11	1,87	12,04*	0,42*	0,00	1,37
	Dolorol	0,08	0,32*	0,52*	0,58*	0,56*	0,49*	0,34*	0,25	0,20	0,16	0,13	0,10	0,08	0,07	0,05	0,04	0,04	0,03	4,12	18,38*	0,24	0,10	4,88
Petersen Paracetamol (B/N 32978)	Pet.(32449)	0,04	0,07	0,04	0,02	0,02	0,02	0,01	0,01	0,01	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,25	0,59	0,02	0,00	0,34
	Panado	0,08	0,37*	0,50*	0,52*	0,50*	0,48*	0,46*	0,43*	0,40*	0,38*	0,35*	0,33*	0,30*	0,28	0,26	0,24	0,22	0,20	6,21	6,90	0,19	0,01	6,75*
	Norstan	0,02	0,17	0,20	0,16	0,11	0,08	0,06	0,05	0,04	0,03	0,03	0,02	0,02	0,02	0,01	0,01	0,01	0,01	0,60	0,51	0,18	0,02	0,90
	Napamol	0,07	0,40*	0,55*	0,58*	0,56*	0,53*	0,49*	0,46*	0,41*	0,37*	0,33*	0,29	0,26	0,23	0,20	0,18	0,16	0,14	6,40*	7,57	0,17	0,11	6,98*
	Dolorol	0,05	0,16	0,11	0,06	0,05	0,03	0,02	0,02	0,02	0,01	0,01	0,01	0,01	0,01	0,00	0,00	0,00	0,00	0,41	1,23	0,01	0,00	0,73
Petersen Paracetamol (B/N 32449)	Panado	0,12*	0,44*	0,53*	0,54*	0,52*	0,50*	0,47*	0,44*	0,41*	0,38*	0,35*	0,33*	0,31*	0,28	0,26	0,24	0,22	0,20	6,46*	7,49	0,17	0,01	7,09*
	Norstan	0,03	0,24*	0,24*	0,18	0,13	0,10	0,07	0,06	0,05	0,04	0,03	0,02	0,02	0,02	0,01	0,01	0,01	0,01	0,85	1,10	0,16	0,02	1,24
	Napamol	0,12*	0,47*	0,59*	0,60*	0,58*	0,54*	0,50*	0,46*	0,42*	0,37*	0,33*	0,30	0,26	0,23	0,20	0,18	0,16	0,14	6,65*	8,16	0,15	0,11	7,32*
	Dolorol	0,09	0,23	0,15	0,09	0,07	0,05	0,04	0,03	0,02	0,02	0,02	0,01	0,01	0,01	0,01	0,00	0,00	0,00	0,66	1,82	0,03	0,01	1,07
Panado	Norstan	0,10	0,20	0,29*	0,36*	0,39*	0,40*	0,40*	0,38*	0,36*	0,34*	0,32*	0,30	0,28	0,27	0,25	0,23	0,21	0,19	5,61	6,40	0,02	0,01	5,85
	Napamol	0,01	0,04	0,05	0,06	0,06	0,04	0,03	0,02	0,01	0,01	0,02	0,03	0,04	0,05	0,06	0,06	0,06	0,05	0,19	0,67	0,02	0,12	0,23
	Dolorol	0,03	0,21	0,39*	0,45*	0,46*	0,45*	0,43*	0,41*	0,39*	0,36*	0,34*	0,32*	0,30*	0,28	0,26	0,24	0,22	0,20	5,80	5,67	0,20	0,02	6,02
Norstan	Napamol	0,09	0,23	0,35*	0,42*	0,45*	0,44*	0,43*	0,41*	0,37*	0,34*	0,30*	0,27	0,24	0,22	0,19	0,17	0,15	0,14	5,80	7,06	0,00	0,13	6,08
	Dolorol	0,07	0,01	0,09	0,09	0,06	0,05	0,04	0,03	0,02	0,02	0,02	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,19	0,73	0,19	0,02	0,17
Napamol	Dolorol	0,02	0,24*	0,44*	0,51*	0,51*	0,49*	0,47*	0,44*	0,40*	0,36*	0,32*	0,29	0,25	0,23	0,20	0,18	0,16	0,14	5,99	6,34	0,18	0,11	6,25*
Range ±		0,11	0,23	0,22	0,22	0,25	0,27	0,28	0,29	0,29	0,29	0,29	0,30	0,29	0,29	0,28	0,28	0,27	0,26	6,28	9,54	0,24	0,13	6,11

APPENDIX 2

TABLE A.2 Results from the Scheffé test - Stationary basket - rotating paddle apparatus

* indicates significant differences at the 95% confidence level.

COMPARISON PAIRS		DIFFERENCE BETWEEN THE MEAN FRACTION DISSOLVED AT EACH TIME INTERVAL (min.)																DISSOLUTION PARAMETERS				
		1,0	2,0	3,0	4,0	5,0	6,0	7,0	8,0	9,0	10,0	11,0	12,0	13,0	14,0	15,0	16,0	$t_{50\%}$	a	b	Ti	Td
Repamol	Propan	0,04	0,14*	0,24*	0,33*	0,41*	0,40*	0,35*	0,30*	0,25*	0,20*	0,16*	0,13*	0,11*	0,08*	0,06	0,04	3,05*	6,07*	0,09	0,03	3,51*
	Pet.(32978)	0,33*	0,62*	0,64*	0,60*	0,54*	0,47*	0,40*	0,33*	0,28*	0,22*	0,18*	0,14*	0,12*	0,09*	0,07*	0,04	4,79*	10,00*	0,12	0,11*	5,53*
	Pet.(32449)	0,39*	0,65*	0,65*	0,61*	0,55*	0,47*	0,40*	0,33*	0,27*	0,22*	0,18*	0,14*	0,12*	0,09*	0,07*	0,04	4,91*	10,18*	0,17	0,10	5,67*
	Panado	0,41*	0,65*	0,66*	0,62*	0,55*	0,47*	0,40*	0,33*	0,27*	0,22*	0,18*	0,14*	0,12*	0,09*	0,07*	0,04	4,92*	10,17*	0,13	0,11*	5,68*
	Norstan	0,26*	0,46*	0,55*	0,57*	0,54*	0,47*	0,40*	0,33*	0,28*	0,22*	0,18*	0,14*	0,12*	0,09*	0,07*	0,04	4,58*	9,26*	0,03	0,12*	5,10*
	Napamol	0,35*	0,51*	0,53*	0,51*	0,46*	0,40*	0,34*	0,28*	0,23*	0,19*	0,15*	0,12*	0,10*	0,07	0,05	0,03	4,81*	9,97*	0,54*	0,15*	5,15*
	Dolorol	0,34*	0,65*	0,66*	0,62*	0,55*	0,47*	0,40*	0,33*	0,27*	0,22*	0,18*	0,14*	0,12*	0,09*	0,07*	0,04	4,81*	10,09*	0,12	0,11*	5,61*
Propan Paracetamol	Pet.(32978)	0,28*	0,48*	0,40*	0,27*	0,13*	0,07	0,05	0,04	0,03	0,02	0,01	0,01	0,01	0,00	0,00	0,00	1,74*	3,93*	0,20	0,08	2,02*
	Pet.(32449)	0,34*	0,51*	0,41*	0,28*	0,14*	0,07	0,05	0,04	0,03	0,02	0,01	0,01	0,01	0,00	0,00	0,00	1,86*	4,11*	0,25	0,07	2,16*
	Panado	0,36*	0,51*	0,42*	0,29*	0,14*	0,08	0,05	0,04	0,03	0,02	0,01	0,01	0,01	0,00	0,00	0,00	1,87*	4,10*	0,21	0,08	2,17*
	Norstan	0,22	0,32*	0,31*	0,24*	0,12*	0,07	0,05	0,04	0,03	0,02	0,01	0,01	0,01	0,00	0,00	0,00	1,53*	3,19*	0,05	0,08	1,59*
	Napamol	0,30*	0,37*	0,29*	0,18*	0,05	0,00	0,01	0,02	0,02	0,02	0,01	0,01	0,01	0,01	0,01	0,01	1,76*	3,90*	0,62*	0,12*	1,55*
	Dolorol	0,29*	0,51*	0,42*	0,29*	0,14*	0,07	0,05	0,04	0,03	0,02	0,01	0,01	0,01	0,00	0,00	0,00	1,76*	4,02*	0,20	0,08	2,10*
Petersen Paracetamol (B/N 32978)	Pet.(32449)	0,06	0,03	0,01	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,12	0,18	0,05	0,01	0,14
	Panado	0,08	0,03	0,02	0,02	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,13	0,16	0,01	0,00	0,15
	Norstan	0,06	0,16*	0,08*	0,03	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,21	0,74	0,15	0,01	0,43
	Napamol	0,02	0,11	0,11*	0,09*	0,08	0,07	0,06	0,05	0,04	0,04	0,03	0,02	0,02	0,02	0,01	0,01	0,02	0,03	0,42*	0,04	0,38
	Dolorol	0,01	0,03	0,02	0,01	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,02	0,09	0,00	0,00	0,08
Petersen Paracetamol (B/N 32449)	Panado	0,02	0,00	0,01	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	0,04	0,01	0,01
	Norstan	0,12	0,19*	0,10*	0,04	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,33	0,92	0,20	0,02	0,57
	Napamol	0,04	0,14*	0,12*	0,10*	0,09*	0,07	0,06	0,05	0,04	0,04	0,03	0,02	0,02	0,02	0,01	0,01	0,10	0,21	0,37*	0,05	0,52
	Dolorol	0,05	0,00	0,01	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,10	0,09	0,05	0,01	0,06
Panado	Norstan	0,14	0,20*	0,11*	0,05	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,34	0,90	0,16	0,00	0,58
	Napamol	0,06	0,14*	0,13*	0,11*	0,09*	0,08	0,06	0,05	0,04	0,04	0,03	0,02	0,02	0,02	0,01	0,01	0,11	0,20	0,41*	0,04	0,53
	Dolorol	0,07	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,11	0,07	0,01	0,00	0,07
Norstan	Napamol	0,08	0,05	0,02	0,07	0,08	0,07	0,06	0,05	0,04	0,04	0,03	0,02	0,02	0,02	0,01	0,01	0,23	0,71	0,57*	0,03	0,05
	Dolorol	0,07	0,19*	0,11*	0,04	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,23	0,83	0,15	0,00	0,51
Napamol	Dolorol	0,01	0,14*	0,13*	0,11*	0,09*	0,07	0,06	0,05	0,04	0,04	0,03	0,02	0,02	0,02	0,01	0,01	0,00	0,12	0,42*	0,04	0,46
Range ±		0,25	0,12	0,06	0,07	0,08	0,08	0,10	0,11	0,12	0,12	0,11	0,10	0,09	0,07	0,06	0,04	1,02	1,72	0,30	0,10	1,42

APPENDIX 3

The 4 x 4 Latin Square analysis of variance had to take into account the fact that a reading was missing in the row total R_i, the column total C_j and the treatment total T_k. When the respective Sum of Squares (SS) were calculated the actual row, column and treatment identities for the missing data were therefore inserted for i, j and k as follows:

$$\text{Row SS} = \frac{R_i^2}{3} + \sum \frac{(\text{other row totals})^2}{4} - \text{c.f.}$$

$$\text{Column SS} = \frac{C_j^2}{3} + \sum \frac{(\text{other column totals})^2}{4} - \text{c.f.}$$

$$\text{Treatment SS} = \frac{T_k^2}{3} + \sum \frac{(\text{other treatment totals})^2}{4} - \text{c.f.}$$

As a result of the missing data point, one degree of freedom is lost overall and there are thus five degrees of freedom for Error.

APPENDIX 4

TABLE A.3 Results of the ANOVA of the *in vivo* parameters

Theoretical F(3,5) = 5,41 at p=0,05

PARAMETER	SOURCE	df	MEAN SQUARE	F
t_{max}	Subject	3	0,124	1,70
	Trial No.	3	0,269	3,68
	Treatment	3	0,353	4,84
	Error	5	0,073	
c_{max}	Subject	3	5,958	1,08
	Trial No.	3	4,863	0,88
	Treatment	3	15,595	2,82
	Error	5	5,524	
$AUC_{0\to 6}$	Subject	3	46,573	4,03
	Trial No.	3	17,992	1,56
	Treatment	3	33,940	2,94
	Error	5	11,564	
$AUC_{0\to\infty}$	Subject	3	36,305	2,68
	Trial No.	3	22,226	1,64
	Treatment	3	44,252	3,27
	Error	5	13,537	
k	Subject	3	0,213	53,25
	Trial No.	3	0,013	3,25
	Treatment	3	0,007	1,75
	Error	5	0,004	
$AUC_{0\to\infty}.k$	Subject	3	49,679	17,58
	Trial No.	3	2,036	0,72
	Treatment	3	4,209	1,49
	Error	5	2,826	

APPENDIX 4 TABLE A.3 continued.

PARAMETER	SOURCE	df	MEAN SQUARE	F
E_2	Subject	3	75,999	25,13
	Trial No.	3	10,761	3,56
	Treatment	3	2,950	0,98
	Error	5	3,024	
E_4	Subject	3	241,192	14,08
	Trial No.	3	36,568	2,13
	Treatment	3	8,318	0,48
	Error	5	17,135	
E_6	Subject	3	437,852	47,91
	Trial No.	3	62,434	6,83
	Treatment	3	10,136	1,11
	Error	5	9,138	
E_8	Subject	3	450,529	48,22
	Trial No.	3	66,625	7,13
	Treatment	3	15,133	1,62
	Error	5	9,344	
E_{12}	Subject	3	386,749	40,42
	Trial No.	3	55,598	5,81
	Treatment	3	11,850	1,24
	Error	5	9,569	
E_{24}	Subject	3	293,129	37,60
	Trial No.	3	49,398	6,34
	Treatment	3	10,917	1,40
	Error	5	7,797	

BIBLIOGRAPHY

1. Martindale, "The Extra Pharmacopoeia", Pharm.Press, London, 27th ed., 1977, page 201.
2. DEARDEN J.C. and TOMLINSON E., J.Pharm.Pharmac., 22, suppl., 53S (1970)
3. SHETH B.B., PARUTA A.N. and NINGER F.C., J.Pharm.Sci., 55, 1144 (1966)
4. GOLDBERG A.H., GIBALDI M. and KANIG J.L., J.Pharm.Sci., 55, 482 (1966)
5. DITTERT L.W., CALDWELL H.C., ADAMS H.J., IRWIN G.M. and SWINTOSKY J.V., J.Pharm.Sci., 57, 774 (1968)
6. SHANE N. and KOWBLANSKY M., J.Pharm.Sci., 57, 1218 (1968)
7. HEADING R.C., NIMMO J., PRESCOTT L.F. and TOTHILL P., Br.J.Pharmac., 47, 415 (1973)
8. CHOW Y.P. and REPTA A.J., J.Pharm.Sci., 61, 1454 (1972)
9. KALATZIS E., J.Pharm.Sci., 59, 193 (1970)
10. KOSHY K.T. and LACH J.L., J.Pharm.Sci., 50, 113 (1961)
11. "The Merck Index", Merck and Co., Inc., Rahway, N.J., U.S.A., 9th ed., 1976, page 6.
12. CHILD K.J., BEDFORD C. and TOMICH E.G., J.Pharm.Pharmac., 14, 374 (1962)
13. WOOD P.R., J.Pharm.Pharmac., 25, 765 (1973)
14. MATTOK G.L., McGILVERAY I.J. and COOK D., Can.J.Pharm.Sci., 6, 35 (1971)
15. RAWLINS M.D., HENDERSON D.B. and HIJAB A.R., Europ.J.Clin.Pharmac., 11, 283, (1977)
16. McGILVERAY I.J. and MATTOK G.L., J.Pharm.Pharmac., 24, 615 (1972)
17. NIMMO W.S., HEADING R.C., WILSON J. and PRESCOTT L.F., Br.Med.J., 2, 1189 (1979)
18. NIMMO W.S. and PRESCOTT L.F., Br.J.Clin.Pharmac., 5, 348 (1978)
19. GWILT J.R., ROBERTSON A., GOLDMAN L. and BLANCHARD A.W., J.Pharm.Pharmac., 15, 445 (1963)
20. WALTERS V., J.Pharm.Pharmac., 20, suppl., 228S (1968)
21. KEINÄNEN S., HIETULA M., SIMILÄ S. and KOUVALAINEN K., Europ.J.Clin.Pharmac., 12, 77 (1977)

22. PAGAY S.N., POUST R.I. and COLAIZZI J.L., J.Pharm.Sci., 63, 44 (1974)

23. MOËS A. and JAMINET F., Pharm.Acta Helv., 51, 119 (1976)

24. GWILT J.R., ROBERTSON A. and McCHESNEY E.W., J.Pharm.Pharmac., 15, 440 (1963)

25. RICHTER A. and SMITH S.E., Br.J.Clin.Pharmac., 1, 495 (1974)

26. PRESCOTT L.F. and WRIGHT N., Br.J.Pharmac., 49, 602 (1973)

27. GAZZARD B.G., FORD-HUTCHINSON A.W., SMITH M.J.H. and WILLIAMS R., J.Pharm.Pharmac., 25, 964 (1973)

28. HUNT C.A. and DUNFORD P.R., J.Am.Pharm.Assoc., NS 17, 517 (1977)

29. DUGGIN G.G. and MUDGE G.H., Br.J.Pharmac., 54, 359 (1975)

30. McGILVERAY I.J., MATTOK G.L., FOOKS J.R., JORDAN N. and COOK D., Can.J.Pharm.Sci., 6, 38 (1971)

31. ALBERT K.S., SEDMAN A.J., WILKINSON P., STOLL R.G., MURRAY W.J. and WAGNER J.G., J.Clin.Pharmac., 14, 264 (1974)

32. WÓJCICKI J., GAWROŃSKA-SZKLARZ B. and KAZIMIERCZYK J., Zbl.Pharm., 116, 355 (1977)

33. HEDGES A., KAYE C.M., MACLAY W.P. and TURNER P., J.Clin.Pharmac., 14, 363 (1974)

34. DECHTLARUK W.A., JOHNSON G.F. and SOLOMON H.M., Clin.Chem., 22, 879 (1976)

35. GLYNN J.P. and BASTAIN W., J.Pharm.Pharmac., 25, 420 (1973)

36. CUMMINGS A.J., KING M.L. and MARTIN B.K., Br.J.Pharmac.Chemother., 29, 150 (1967)

37. FULTON B., JAMES O. and RAWLINS M.D., Br.J.Clin.Pharmac., 7, 418 (1979)

38. CLEMENTS J.A. and PRESCOTT L.F., J.Pharm.Pharmac., 28, 707 (1976)

39. PERUCCA E. and RICHENS A., Br.J.Clin.Pharmac., 7, 201 (1979)

40. LEVY G. and YAMADA H., J.Pharm.Sci., 60, 215 (1971)

41. MITCHELL J.R., THORGEIRSSON S.S., POTTER W.Z., JOLLOW D.J. and KEISER H., Clin.Pharmac.Ther., 16, 676 (1974)

42. HOWIE D., ADRIAENSSENS P.I. and PRESCOTT L.F., J.Pharm.Pharmac., 29, 235 (1977)

43. AMSEL L.P. and DAVISON C., J.Pharm.Sci., 61, 1474 (1972)

44. NELSON E. and MORIOKA T., J.Pharm.Sci., 52, 864 (1963)

45. LEVY G. and REGÅRDH C., J.Pharm.Sci., 60, 608 (1971)

46. CHIOU W.L., J.Pharm.Sci., 64, 1734 (1975)

47. DAVIDSON D.G.D. and EASTHAM W.N., Br.Med.J., 2, 497 (1966)

48. KNOX J.H. and JURAND J., J.Chromatogr., 149, 297 (1978)

49. PRESCOTT L.F., WRIGHT N., ROSCOE P. and BROWN S.S., Lancet, i, 519 (1971)

50. MITCHELL J.R., JOLLOW D.J., POTTER W.Z., DAVIS D.C., GILLETTE J.R. and BRODIE B.B., J.Pharmac.Exp.Ther., 187, 185 (1973)

51. JOLLOW D.J., MITCHELL J.R., POTTER W.Z., DAVIS D.C., GILLETTE J.R. and BRODIE B.B., J.Pharmac.Exp.Ther., 187, 195 (1973)

52. POTTER W.Z., DAVIS D.C., MITCHELL J.R., JOLLOW D.J., GILLETTE J.R. and BRODIE B.B., J.Pharmac.Exp.Ther., 187, 203 (1973)

53. MITCHELL J.R., JOLLOW D.J., POTTER W.Z., GILLETTE J.R. and BRODIE B.B., J.Pharmac.Exp.Ther., 187, 211 (1973)

54. GROVER P.L. and SIMS P., Biochem.J., 90, 603 (1964)

55. PRESCOTT L.F., NEWTON R.W., SWAINSON C.P., WRIGHT N., FORREST A.R.W. and MATTHEW H., Lancet, i, 588 (1974)

56. PRESCOTT L.F., PARK J., SUTHERLAND G.R., SMITH I.J. and PROUDFOOT A.T., Lancet, ii, 109 (1976)

57. CROME P., VALE J.A., VOLANS G.N., WIDDOP B. and GOULDING R., Lancet, ii, 829 (1976)

58. MAURER W.G. and ZEISLER J., Am.J.Hosp.Pharm., 35, 1025 (1978)

59. MARQUARDT E.D., Am.J.Hosp.Pharm., 34, 805 (1977)

60. SMITH J.M., ROBERTS W.O., HALL S.M., WHITE T.A. and GILBERTSON A.A., Br.Med.J., 1, 331 (1978)

61. BUCHANAN N. and MOODLEY G.P., Br.Med.J., 2, 307 (1979)

62. WELCH R.M. and CONNEY A.H., Clin.Chem., 11, 1064 (1965)

63. ANON., Bulletin of the Poison Control Center, University of Utah College of Pharmacy, 2, 1 (1974)

64. CAMPAGNA F.A., CURETON G., MIRIGIAN R.A. and NELSON E., J.Pharm.Sci., 52, 605 (1963)

65. BARR W.H., Drug Inform.Bull., 3, 27 (1969)

66. PRESCOTT L.F. and NIMMO J., Acta Pharmac.et Toxicol., 29, 288 (1971)

67. SCHNELLER G.H., J.Am.Pharm.Assoc., NS 9, 455 (1969)

68. DITTERT L.W. and DISANTO A.R., in "The Bioavailability of Drug Products", American Pharmaceutical Association, Washington, D.C., 1975, page 5.

69. HERSEY J.A., Mfg.Chem.Aerosol News, 40, 32 (1969)

70. LERK C.F., Pharm.Weekblad, 111, 1157 (1976)

71. COOPER J. and REES J.E., J.Pharm.Sci., 61, 1511 (1972)

72. HERSEY J.A. and MARTY J.J., Mfg.Chem.Aerosol News, 46, 43 (1975)

73. PERNAROWSKI M., in "Dissolution Technology", edited by Leeson L.J. and Carstensen J.T., The Industrial Pharmaceutical Technology Section of the Academy of Pharmaceutical Science, Washington, D.C., 1974, Chapter 3, page 58.

74. WITHEY R.J., J.Pharm.Pharmac., 23, 573 (1971)

75. OGATA H., SHIBAZAKI T., INOUE T. and EJIMA A., J.Pharm.Sci., 68, 708 (1979)

76. GIBALDI M. and FELDMAN S., J.Pharm.Sci., 56, 1238 (1967)

77. TINGSTAD J.E. and RIEGELMAN S., J.Pharm.Sci., 59, 692 (1970)

78. GIBALDI M. and WEINTRAUB H., J.Pharm.Sci., 59, 725 (1970)

79. HIGUCHI W.I., ROWE E.L. and HIESTAND E.N., J.Pharm.Sci., 52, 162 (1963)

80. HAMLIN W.E., NELSON E., BALLARD B.E. and WAGNER J.G., J.Pharm.Sci., 51, 432 (1962)

81. BROADHEAD J.F., MITCHELL A.G. and O'REILLY W.J., Austral.J.Pharm., 47 (562), S92 (1966)

82. HUBER H.E., DALE L.B. and CHRISTENSON G.L., J.Pharm.Sci., 55, 974 (1966)

83. KAPLAN L.L., J.Pharm.Sci., 54, 457 (1965)

84. SCHROETER L.C. and HAMLIN W.E., J.Pharm.Sci., 52, 811 (1963)

85. LAZARUS J., PAGLIERY M. and LACHMAN L., J.Pharm.Sci., 53, 798 (1964)

86. CALDWELL H.C., J.Pharm.Sci., 63, 770 (1974)

87. TIMKO R.J. and LORDI N.G., J.Pharm.Sci. 67, 496 (1978)

88. MAEDA T., TAKENAKA H., YAMAHIRA Y. and NOGUCHI T., J.Pharm.Sci., 68, 1286 (1979)

89. LANGENBUCHER F., J.Pharm.Sci., 58, 1265 (1969)

90. BAUN D.C. and WALKER G.C., J.Pharm.Sci., 58, 611 (1969)

91. TINGSTAD J., GROPPER E., LACHMAN L. and SHAMI E., J.Pharm.Sci., 61, 1985 (1972)

92. SMOLEN V.F. and WEIGAND W.A., J.Pharm.Sci., 65, 1718 (1976)

93. RIPPIE E.G. and HUQ A.S.M.S., J.Pharm.Sci., 68, 938 (1979)

94. "The United States Pharmacopoeia XX National Formulary XV", United States Pharmacopoeial Convention, Inc., Rockville, Md., 1979, page 959.

95. CARSTENSEN J.T., LAI T.Y. and PRASAD V.K., J.Pharm.Sci., 67, 1303 (1978)

96. PERNAROWSKI M., WOO W. and SEARL R.O., J.Pharm.Sci., 57, 1419 (1968)

97. ALLEN P.V., RAHN P.D., SARAPU A.C. and VANDERWIELEN A.J., J.Pharm.Sci., 67, 1087 (1978)

98. LEVY G. and HAYES B.A., New Engl.J.Med., 262, 1053 (1960)

99. LERK C.F. and LAGAS M., Pharm.Weekblad, 112, 925 (1977)

100. BEYER W.F. and SMITH E.W., J.Pharm.Sci., 60, 1556 (1971)

101. KENT J.S., WONG P.P. and HEGDE G.P., J.Pharm.Sci., 66, 1665 (1977)

102. RICHTER A., MYHRE B. and KHANNA S.C., J.Pharm.Pharmac., 21, 409 (1969)

103. JOHANSSON R., REGÅRDH C.G. and SJÖGREN J., Acta Pharm.Suecica, 8, 59 (1971)

104. FRASER E.J., LEACH R.H., POSTON J.W., BOLD A.M., CULANK L.S. and LIPEDE A.B., J.Pharm.Pharmac., 25, 968 (1973)

105. SIRENIUS I., KROGERUS V.E. and LEPPÄNEN T., J.Pharm.Sci., 68, 791 (1979)

106. NEWTON J.M. and BADER F., J.Pharm.Pharmac., 32, 167 (1980)

107. CHOWHAN Z.T., J.Pharm.Pharmac., 32, 10 (1980)

108. PILBRANT Å., GLENNE P., SUNDWALL A., VESSMAN J. and WRETLIND M., Acta Pharmac. et Toxicol., 40, suppl. I, 7 (1977)

109. NEEDHAM T.E., SHAH K., KOTZAN J. and ZIA H., J.Pharm.Sci., 67, 1070 (1978)

110. FELL J.T., CALVERT R.T. and RILEY-BENTHAM P., J.Pharm.Pharmac., 30, 479 (1978)

111. MILSAP R.L., AYRES J.W., MACKICHAN J.J. and WAGNER J.G., Biopharm.Drug Dispos., 1, 3 (1979)

112. STUPAK E.I. and BATES T.R., J.Pharm.Sci., 61, 400 (1972)

113. WEINTRAUB H. and GIBALDI M., J.Pharm.Sci., 59, 1792 (1970)

114. LEVY G., J.Pharm.Sci., 52, 1039 (1963)

115. LEVY G. and HOLLISTER L.E., J.Pharm.Sci., 53, 1446 (1964)

116. LAGAS M. and LERK C.F., Pharm.Weekblad, 112, 845 (1977)

117. FELL J.T., CALVERT R.T. and RILEY-BENTHAM P., Expo.Congress on International Technology and Pharmaceutics, 1st, 5, 121 (1977)

118. LERK C.F., LAGAS M., LIE-A-HUEN L., BROERSMA P. and ZUURMAN K., J.Pharm.Sci., 68, 634 (1979)

119. MATTOK G.L., McGILVERAY I.J. and HOSSIE R.D., J.Pharm.Sci., 61, 460 (1972)

120. BEYER W.F. and SMITH D.L., J.Pharm.Sci., 60, 496 (1971)

121. ROSOLIA A.M., O'CONNELL J.R., BAVITZ J.F., RESTAINO F.A. and SCHWARTZ J.B., J.Pharm.Sci., 61, 1638 (1972)

122. CARSTENSEN J.T., LAI T. and PRASAD V.K., J.Pharm.Sci., 66, 607 (1977)

123. LAGAS M. and LERK C.F., Pharm.Weekblad, 113, 1277 (1978)

124. WITHEY R.J. and BOWKER A.J., J.Pharm.Pharmac., 24, 345 (1972)

125. CARSTENSEN J.T., KOTHARI R., PRASAD V.K. and SHERIDAN J., J.Pharm.Sci., 69, 290 (1980)

126. UNDERWOOD T.W. and CADWALLADER D.E., J.Pharm.Sci., 61, 239 (1972)

127. LIN S., MENIG J. and SWARTZ C.J., J.Pharm.Sci., 59, 989 (1970)

128. SARAPU A.C. and CLARK L., J.Pharm.Sci., 69, 129 (1980)

129. "British Pharmacopoeia", Her Majesty's Stationery Office, London, 1973, page 341.

130. GANDERTON D., HADGRAFT J.W., RISPIN W.T. and THOMPSON A.G., Pharm.Acta Helv., 42, 152 (1967)

131. GOOSENS A.P.G., "Die *in vitro* en *in vivo* bepaling van die vrystelling van enkele sulfonamiede uit tablette.", D.Sc.(Pharm.), Thesis, University of Potchefstroom (CHE), Potchefstroom, S.A., 1971.

132. KATCHEN B. and SYMCHOWICZ S., J.Pharm.Sci., 56, 1108 (1967)

133. CLARKE J.M., RAMSAY L.E., SHELTON J.R., TIDD M.J., MURRAY S. and PALMER R.F., J.Pharm.Sci., 66, 1429 (1977)

134. MENDES R.W., MASIH S.Z. and KANUMURI R.R., J.Pharm.Sci., 67, 1616 (1978)

135. KLINK P.R., POUST R.I., COLAIZZI J.L. and McDONALD R.H., J.Pharm.Sci., 63, 1231 (1974)

136. YLITALO P., WILÉN G. and LUNDELL S., J.Pharm.Sci., 64, 1264 (1975)

137. STOLL R.G., CHAO G.C., HOYT H.J. and YACOBI A., J.Pharm.Sci., 67, 1328 (1978).

138. CRESSMAN W.A., JANICKI C.A., JOHNSON P.C., DOLUISIO J.T. and BRAUN G.A., J.Pharm.Sci., 58, 1516 (1969)

139. WOOD J.H., Pharm.Acta Helv., 42, 129 (1967)

140. WAGNER J.G., in "Biopharmaceutics and Relevant Pharmacokinetics", Drug Intelligence Publications, Hamilton, Illinois, 1st ed., 1971, chapter 16, page 104.

141. Ibid., chapter 18, page 115.

142. KITAZAWA S., JOHNO I., MINOUCHI T. and OKADA J., J.Pharm.Pharmac., 29, 453 (1977)

143. BEAUBIEN L.J. and VANDERWIELEN A.J., J.Pharm.Sci., 69, 651 (1980)

144. LANGENBUCHER F., J.Pharm.Pharmac., 24, 979 (1972)

145. NELDER J.A. and MEAD R., Computer J., 7, 308 (1965)

146. PEDERSEN P.V. and MYRICK J.W., J.Pharm.Sci., 67, 1450 (1978)

147. KANFER I., School of Pharmacy, Dept. of Pharmacy, University of California, San Francisco, California, Personal communication.

148. MORONEY M.J., in "Facts from Figures", Penguin Books Ltd., Harmondsworth, Middlesex, England, 2nd ed., 1953, chapter 19, page 371.

149. KOCH G.S. and LINK R.F., in "Statistical Analysis of Geological Data", John Wiley and Sons, Inc., New York, 1970, page 196.

150. BRODIE B.B. and AXELROD J., J.Pharmac.Exp.Ther., 94, 22 (1948)

151. ROUTH J.I., SHANE N.A., ARREDONDO E.G. and PAUL W.D., Clin.Chem., 14, 882 (1968)

152. DITTERT L.W., ADAMS H.J., ALEXANDER F., CHONG C.W., ELLISON T. and SWINTOSKY J.V., J.Pharm.Sci., 57, 1146 (1968)

153. PLAKOGIANNIS F.M. and SAAD A.M., J.Pharm.Sci., 67, 581 (1978)

154. GLYNN J.P. and KENDAL S.E., Lancet, i, 1147 (1975)

155. WIENER K., Ann.Clin.Biochem., 14, 55 (1977)

156. PRESCOTT L.F., J.Pharm.Pharmac., 23, 111 (1971)

157. GROVE J., J.Chromatogr., 59, 289 (1971)

158. LINDLEY T.N. and SHARMAN J.R., N.Z.J.Med.Lab.Technol., 31, 71 (1977)

159. THOMAS B.H. and COLDWELL B.B., J.Pharm.Pharmac., 24, 243 (1972)

160. PRESCOTT L.F., J.Pharm.Pharmac., 23, 807 (1971)

161. STAVCHANSKY S., WU P. and KOSTENBAUDER H.B., Anal.Letters, 10, 845 (1977)

162. HACKETT L.P. and DUSCI L.J., Clin.Chim.Acta, 74, 187 (1977)

163. THOMA J.J., McCOY M., EWALD T. and MYERS N., J.Anal.Toxicol., 2, 226 (1978).

164. EVANS M.A. and HARBISON R.D., J.Pharm.Sci., 66, 1628 (1977)

165. SERFONTEIN W.J., COETZEE M., DE VILLIERS L.S. and BOTHA D., S.A.J.Med.Sci., 41, 297 (1976)

166. RIGGIN R.M., SCHMIDT A.L. and KISSINGER P.T., J.Pharm.Sci., 64, 680 (1975)

167. WONG L.T., SOLOMONRAJ G. and THOMAS B.H., J.Pharm.Sci., 65, 1064, (1976)

168. GOTELLI G.R., KABRA P.M. and MARTON L.J., Clin.Chem., 23, 957 (1977)

169. HORVITZ R.A. and JATLOW P.I., Clin.Chem., 23, 1596 (1977)

170. MINER D.J. and KISSINGER P.T., J.Pharm.Sci., 68, 96 (1979)

171. FLETTERICK C.G., GROVE T.H. and HOHNADEL D.C., Clin.Chem. 25, 409 (1979)

172. MICELI J.N., ARAVIND M.K., COHEN S.N. and DONE A.K., Clin.Chem., 25, 1002 (1979)

173. BUCKPITT A.R., ROLLINS D.E., NELSON S.D., FRANKLIN R.B. and MITCHELL J.R., Anal.Biochem., 83, 168 (1977)

174. KNOX J.H. and JURAND J., J.Chromatogr., 142, 651 (1977)

175. ADRIAENSSENS P.I. and PRESCOTT L.F., Br.J.Clin.Pharmac., 6, 87 (1978)

176. BUCHANAN T., ADRIAENSSENS P. and STEWART M.J., Clin.Chim.Acta, 99, 161 (1979)

177. MROCHEK J.E., KATZ S., CHRISTIE W.H. and DINSMORE S.R., Clin.Chem., 20, 1086 (1974)

178. BURTIS C.A., BUTTS W.C. and RAINEY W.T., Am.J.Clin.Path., 53, 769 (1970)

179. BLAIR D. and RUMACK B.H., Clin.Chem., 23, 743 (1977)

180. MAJORS R.E., J.Chromatogr.Sci., 15, 334 (1977)

181. COHEN J.L., in "Analytical Profiles of Drug Substances", vol. 4, edited by Florey K., Academic Press, Inc., New York, 1975, page 479.

182. PEDERSEN P.V., J.Pharm.Sci., 69, 318 (1980)

183. RITSCHEL W.A., in "Handbook of Basic Pharmacokinetics", Drug Intelligence Publications, Hamilton, Illinois, 1st ed., 1976, chapter 21, page 240.

184. WAGNER J.G., Drug Inform.Bull., 3, 45 (1969)

185. CLEMENTS J.A., HEADING R.C., NIMMO W.S. and PRESCOTT L.F., Clin.Pharmac.Ther., 24, 420 (1978)