THE FORMULATION AND EVALUATION OF ALCOHOL-FREE PAEDIATRIC PARACETAMOL PREPARATIONS

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Dissertation submitted in partial fulfillment of the requirements for the degree Magister Scientiae in the Department of Pharmaceutics, School of Pharmacy, at the Potchefstroom Campus of North-West University.

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POTCHEFSTROOM

2005

To my wonderful loving parents and grandmother

ACKNOWLEDGEMENTS

I would like to express my gratitude and most sincere appreciation to those who have made contributions to my work and all the wonderful people who have supported me throughout:

- The Department of Pharmaceutics, School of Pharmacy, at the Potchefstroom Campus of North-West University for allowing me to further my studies.
- The Research Institute for Industrial Pharmacy, for the use of their chemicals, equipment and facilities.
- The National Research Foundation, for financial assistance.
- Dr. E. Swanepoel, my supervisor, for her guidance, knowledgeable advice and all the hours spent proof-reading my work.
- Prof. A.P. Lötter, my co-supervisor, for his expertise and invaluable assistance during formulation.
- Prof. W. Liebenberg, for her kindness, support and encouragement.
- Ms. J. Handford, for her support and revision of the grammar and style of this dissertation.
- Mariena Nieman, for her patient help and advice with the troublesome HPLC assays.
- Susan May, for performing the viscosity tests.
- Corné Fourie and Charlina Clack, for their help with the stability program.
- My colleagues and fellow students, for their friendship and interest, and for making the RIIP such a wonderful place to work.
- My friends, Annésta, Anita, Jana, Julia, Marga, Mia, Nelia and Wilna, for their support and encouragement.
- My parents, for their love and emotional and financial support. Thank you for always believing in me and granting me this wonderful opportunity.
- My extraordinary grandmother, for her love, support and wisdom. I feel blessed to be able share this with her.

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ABSTRACT

Paracetamol is currently the analgesic / antipyretic which is most commonly used. It is inexpensive, available without prescription and is relatively safe in therapeutic doses. Since toxic effects are so rare, paracetamol is the drug of choice for treating pain and fever in children. The poor water-solubility of paracetamol, as well as its bitter taste, complicates the formulation of oral paediatric preparations.

It has been shown that ethanol increases the risk of paracetamol-induced hepatotoxicity and that its use should be avoided. Various co-solvents were considered as alternative to ethanol for use in aiding the solubility of paracetamol. Six different products were formulated and evaluated.

Visual assessment of the samples revealed that only the suspensions stored at 40°C ± 2°C / 75% RH ± 5% RH underwent a change in appearance.

Altered versions of validated HPLC methods were used to determine the paracetamol and 4-aminophenol content of the six products. The changes to the methods were essential to remove xanthan gum from the test samples, but gave rise to an unavoidable complication during sample preparation. If not removed from the samples, xanthan gum was found to accumulate in the column.

The results obtained from the paracetamol assay weren't reproducible and variation between duplicate samples was substantial in many instances.

Though no definite conclusion as to the stability regarding paracetamol content can be made without accurate HPLC results, other tests indicate that the products formulated were stable at room temperature. Furthermore, it could not be shown that either halving the conventional paracetamol concentration (2.4 g / 100 ml), or adding 0.5% v/v ethanol to a formulation had any beneficial effects on product stability. Thus, formulating alcohol-free 2.4 g / 100 ml paracetamol solutions and suspensions can be achieved by using alternative co-solvents in amounts not exceeding their recommended concentrations.

UITTREKSEL

Parasetamol is tans die pynstiller / koorsweerder wat mees algemeen gebruik word. Dit is bekostigbaar, beskikbaar sonder voorskrif en is relatief veilig in terapeutiese dosisse. Aangesien newe-effekte so selde voorkom, is parasetamol die voorkeur middel in die behandeling van pyn en koors by kinders. Die swak wateroplosbaarheid van parasetamol, asook sy bitter smaak, kompliseer die formulering van orale pediatriese middels.

Dit is bewys dat etanol die risiko van parasetamol geïnduseerde hepatotoksisiteit verhoog en dat die gebruik daarvan vermy behoort te word. Verskeie hulp-oplosmiddels is oorweeg as alternatief vir etanol om die oplosbaarheid van parasetamol te verbeter. Ses verskillende produkte is geformuleer en geëvalueer.

Visuele beoordeling van die produkte het gewys dat slegs die suspensies wat bewaar is by 40°C ± 2°C / 75% RH ± 5% RH verandering in voorkoms ondergaan het.

Gewysigde weergawes van gevalideerde HPLC metodes is gebruik om die parasetamol en 4-aminofenol inhoud van die ses produkte te bepaal. Die wysigings tot die metodes was noodsaaklik om xanthan gom uit die monsters te verwyder, maar het aanleiding gegee tot 'n onvermydelike komplikasie gedurende monstervoorbereiding. Indien xanthan gom nie uit die monsters verwyder word nie, is gevind dat dit in die kolom akkumuleer.

Die parasetamol bepaling se resultate was nie herhaalbaar nie en die variasie tussen duplikaat monsters was in sommige gevalle noemenswaardig.

Hoewel geen definitiewe afleiding omtrent stabiliteit aangaande parasetamol inhoud gemaak kan word sonder akkurate HPLC resultate nie, dui ander toetse daarop dat die produkte wat geformuleer is, wel stabiel is by kamertemperatuur. Verder kon dit nie bewys word dat die stabiliteit bevoordeel word deur 0.5% v/v etanol by te voeg, of deur die konvensionele parasetamol konsentrasie (2.4 g / 100 ml) te halveer nie. Dus, dit is moontlik om alkoholvrye 2.4 g / 100 ml parasetamol oplossings en suspensies te formuleer deur gebruik te maak van alternatiewe hulp-oplosmiddels in hoeveelhede wat nie hul voorgeskrewe konsentrasies oorskry nie.

CHAPTER 1

PARACETAMOL

1.1. INTRODUCTION

Paracetamol is the most commonly used analgesic / antipyretic of recent years. It is available without prescription under well known brand names such as Calpol®, Tylenol®, Panadol® / Panado® and others. Although its analgesic and antipyretic action is comparable to that of aspirin, it is a poor anti-inflammatory. Despite this, paracetamol remains a popular alternative to aspirin, because it is does not pose the same risk for gastric ulceration. Paracetamol is well-tolerated in therapeutic doses and is therefore widely used in paediatrics. In cases of acute or chronic overdose, paracetamol may cause irreversible liver and kidney damage. This is especially true in cases where alcohol is involved (Hardman & Limbird, 1996:631; Lund, 1994:988).

1.2. ORIGIN AND HISTORY

Acetanilide is the parent member of the *p*-aminophenol group of drugs. Its antipyretic and analgesic properties were discovered by Arnold Cahn and Paul Hepp quite accidentally. Drs. Cahn and Hepp administered what they believed was naphthalene to a patient to rid him of intestinal parasites. The patient was also suffering from a febrile disease and although the parasite was unfazed, a marked reduction in fever was observed. Fortunately the substance was analysed when Cahn and Hepp noted the absence of naphthalene's characteristic "moth-ball" odour. The antipyretic drug was in fact acetanilide. It was subsequently introduced into medicine in 1886 under the name of antifebrin, but soon proved to be excessively toxic (Hardman & Limbird, 1996:631; Knoerzer, 2002:5; Nancy West Communications, 2004:4-5).

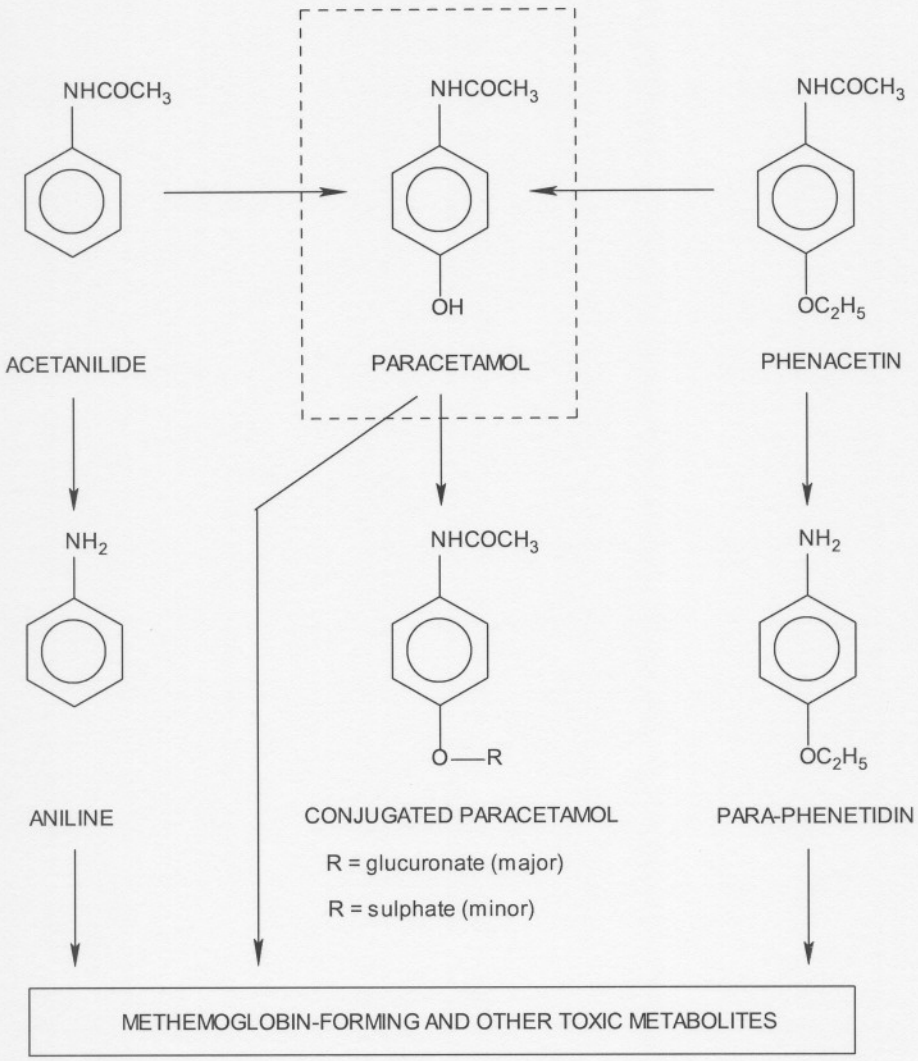
In the search for less toxic compounds, *p*-aminophenol and a number of its derivatives were tested. One of the more satisfactory of these was phenacetin (acetophenetidin). It was discovered by Carl Duisberg (figure 1), who was at that time the director of research for the Friedrich Bayer Company. Bayer produced large

amounts of *p*-aminophenol (figure 2) as a by-product of dye manufacture (Hardman & Limbird, 1996:631; Knoerzer, 2002:5; Nancy West Communications, 2004:4-5).

Figure 1: Carl Duisberg

(Bayer 2004:1)

Figure 2: Para-aminophenol



Scheme 1: Para-aminophenol derivatives and their interrelations

(Hardman & Limbird, 1996:631)

After reading about antifebrin, Duisberg reasoned that a compound with a similar molecular structure might have the same therapeutic uses. He proceeded to mask the hydroxyl group of *p*-aminophenol with an ethyl substituent and then added an acetyl group to produce phenacetin. The drug has been extensively employed in analgesic products since 1887, but due to its toxicity phenacetin is no longer used in analgesic or antipyretic medicines (Hardman & Limbird, 1996:631; Knoerzer, 2002:5; Nancy West Communications, 2004:4-5).

Paracetamol was first used in medicine by Joseph von Mering as early as 1893, but has only gained popularity since 1949 after it was shown to be the major active metabolite of both acetanilide and phenacetin (scheme 1) (Hardman & Limbird, 1996:631).

1.3. STRUCTURE AND PHYSICO-CHEMICAL PROPERTIES

1.3.1. STRUCTURE AND SYNONYMS

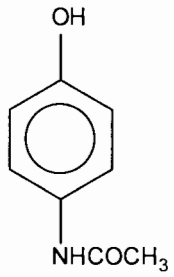


Figure 3: Paracetamol (Lund, 1994:987)

As can be seen in figure 3, paracetamol is a *p*-aminophenol derivative and its synonyms are as follows:

Acetaminophen; *N*-acetyl-*p*-aminophenol; 4'-hydroxyacetanilide; (4-hydroxyphenyl)acetamide; *p*-acetamidophenol; *p*-acetaminophenol; *p*-hydroxyacetanilide; *p*-acetylaminophenol; *N*-*p*-hydroxyphenylacetamide (Lund, 1994:987; El-Obeid & Al-Badr, 1985:552; Martindale, 1993:27).

1.3.2. PHYSICAL PROPERTIES

Paracetamol is an odourless white crystalline powder with a bitter taste. Its physical properties are summarized in table 1.

Table 1: Physical properties of paracetamol

(Lund, 1994:987-988)

Empirical formula	$C_8H_9NO_2$
Molecular mass	151.2
Melting point	168°C - 172°C
pH of a saturated solution	5.3 – 6.5
Dissociation constant	pKa 9.5 (25°C)
Solubility	
- in cold water	1 in 70
- in boiling water	1 in 20
- in ethanol	1 in 7
- in acetone	1 in 13
- in glycerol	1 in 40
- in propylene glycol	1 in 9

Paracetamol is also soluble in methanol, dimethylformamide, ethylene dichloride, ethyl acetate and in solutions of alkali hydroxides (Lund, 1994:987-988; Martindale, 1993:27).

1.3.3. STABILITY AND STORAGE

1.3.3.1. Solid state

Pure, dry paracetamol is stable at temperatures of up to 45°C and relatively stable to oxidation. Paracetamol absorbs insignificant amounts of moisture at 25°C and relative humidities of up to 90%, but exposure to humid conditions will cause hydrolysis to *p*-aminophenol and acetic acid. *p*-aminophenol degrades by oxidation

to quinonimine and other coloured products that may be pink, brown or black (Lund, 1994:988-989; Martindale, 1993:27).

Paracetamol should be kept in tight, light-resistant containers.

1.3.3.2. Solutions

The hydrolysis of paracetamol is a first-order reaction and is both acid-catalysed and base-catalysed. The degradation rate is directly dependent on the paracetamol concentration, but is also a function of pH (table 2). Hydrolysis is at a minimum within the pH range 5 to 6 (Lund, 1994:988-989; Koshy & Lach, 1961:113-118).

Table 2: Half-life of paracetamol as a function of pH at 25°C

(Koshy & Lach, 1961:117)

pH	Half-life (years)
2	0.73
3	5.83
4	15.39
5	19.78
6	21.80
7	12.59
8	7.13
9	2.28

Paracetamol solutions should be protected from light.

1.3.3.3. Suspensions

Solubility of paracetamol in water increases with an increase in temperature (Lund, 1994:988; Martindale, 1993:27), therefore it can be reasoned that suspensions of paracetamol in aqueous media should be protected from high temperatures lest the suspended particles should go into solution.

Paracetamol suspensions should be stored below 25°C in light-resistant containers.

1.4. PHARMACOLOGICAL PROPERTIES

Paracetamol has analgesic and antipyretic effects similar to that of aspirin, but has negligible anti-inflammatory effects. This lack of anti-inflammatory action is yet to be fully understood, but it is thought that paracetamol is a weak inhibitor of cyclo-oxygenase in the presence of the high concentrations of peroxides found in inflammatory lesions. Also, paracetamol does not inhibit neutrophil activation (Hardman & Limbird, 1996:631).

Therapeutic doses of paracetamol have no effect on the following (Hardman & Limbird, 1996:631):

- Cardiovascular and respiratory systems;
- Acid-base balance;
- Platelets and bleeding time;
- Excretion of uric acid; and
- Occurrence of gastric irritation, erosion and bleeding.

1.5. THERAPEUTIC USES

Paracetamol is indicated for the relief of pyrexia and mild to moderate pain, such as headache and myalgia. It is useful as an analgesic adjunct to anti-inflammatory therapy in cases of inflammatory conditions such as rheumatoid arthritis. Paracetamol does not antagonise the effects of uricosuric agents and may be used along with probenecid in the treatment of gout. It is widely used as an alternative to aspirin where the use of the latter may be contra-indicated; aspirin allergy, haemophilia, history of peptic ulcers and where bronchospasm is precipitated by aspirin. In children with viral conditions paracetamol is the drug of choice for the relief of pain and fever, because of the implication of aspirin in the increased incidence of Reye's syndrome (Hardman & Limbird, 1996:632; Katzung, 2001:615; Spraycar, 1995:1739).

The normal oral adult dose is 0.5-1.0 g, 4-6 hourly as required with a maximum of 4 g in 24 hours. The recommended oral paediatric dose is 10 mg/kg/dose, 6-8 hourly (Gibbon, 2000:369). Table 3 gives approximate paediatric doses correlating to the age, and thus the expected weight, of the child.

Table 3: Paediatric paracetamol dosage (Gibbon, 2000:369)

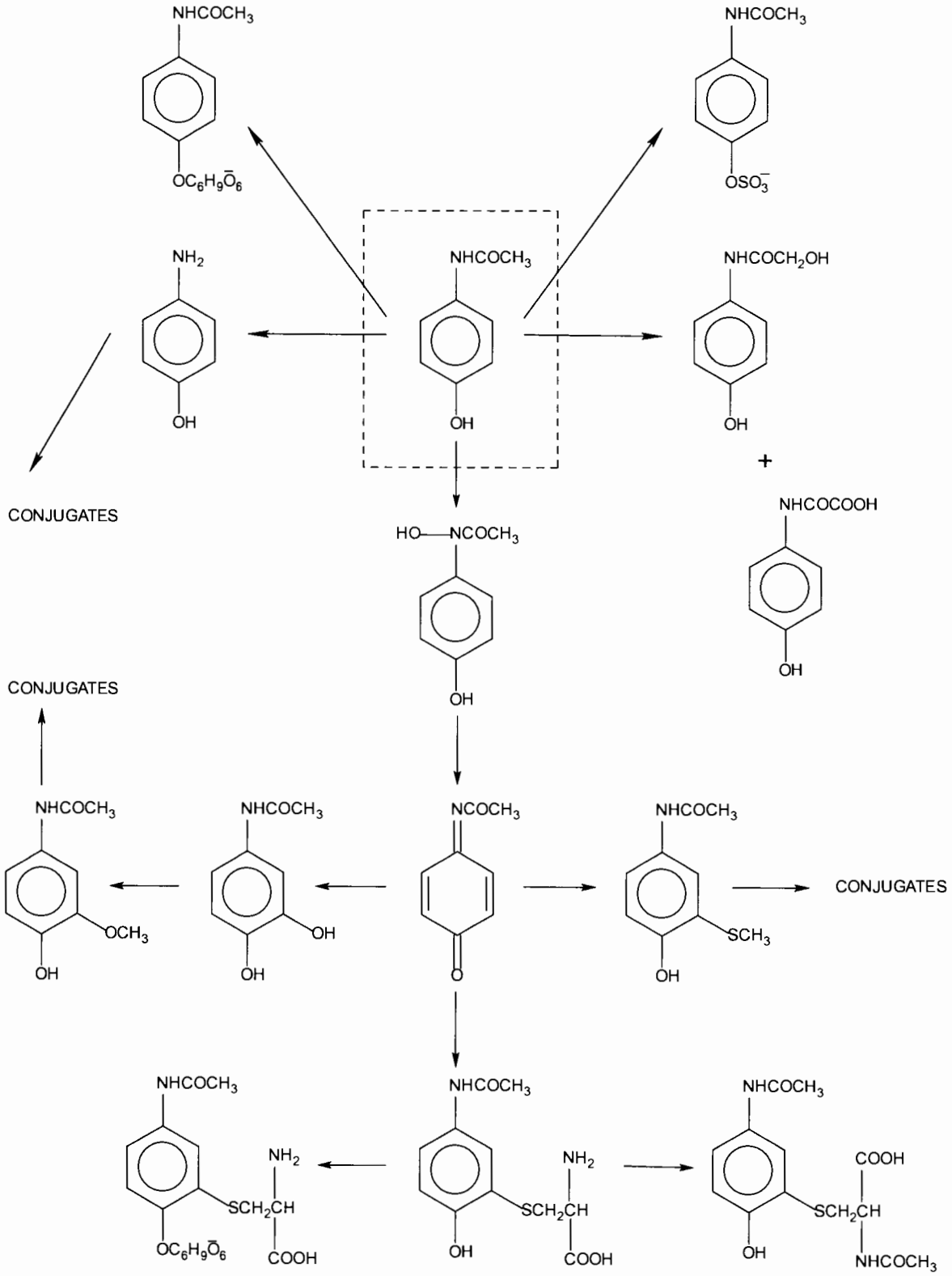
Age of Child	**Oral Dose (6-8 hourly)**	**Rectal Dose (8-12 hourly)**
3-12 months	60-120 mg	125 mg
1-5 years	120-250 mg	250 mg
6-12 years	250-500 mg	-

1.6. PHARMACOKINETICS, METABOLISM AND TOXICITY

1.6.1. PHARMACOKINETICS

Paracetamol is rapidly and almost completely absorbed from the gastrointestinal tract after oral administration. It is distributed relatively uniformly throughout most body fluids and also crosses the placenta. Peak drug plasma levels are reached within 30-60 minutes and the half-life in plasma is on average about 2 hours following a therapeutic dose. The half-life can be greatly increased in cases of hepatic insufficiency or overdose. Variations in the amount of paracetamol absorbed occur depending on the vehicle used and the route of administration. Oral formulations produce greater serum levels than commercially available polyethylene glycol suppository formulations; therefore equivalent mg/kg doses would not be feasible (El-Obeid & Al-Badr, 1985:564; Hardman & Limbird, 1996:632).

1.6.2. METABOLISM



Scheme 2: Metabolites of paracetamol (El-Obeid & Al-Badr, 1985:565)

Following therapeutic doses, 25% of the paracetamol administered is metabolised upon the first pass through the liver. The drug is largely excreted by the kidneys as various conjugates (scheme 2) produced by the liver (El-Obeid & Al-Badr, 1985:564; Hardman & Limbird, 1996:632):

- Glucuronide conjugates (60%);
- Sulphate conjugates (35%); and
- Cysteine and mercapturic acid conjugates (3%).

Small amounts of hydroxylated and deacetylated metabolites have been detected. Approximately 2% of the administered dose is excreted unchanged, though this may increase to 10-14% after an overdose (El-Obeid & Al-Badr, 1985:564; Hardman & Limbird, 1996:632).

1.6.3. ADVERSE EFFECTS AND CONTRA-INDICATIONS

1.6.3.1. Adverse effects

At therapeutic doses paracetamol is generally well-tolerated and the following adverse effects rarely occur:

- Erythematous or urticarial skin rash;
- Drug fever;
- Mucosal lesions; and
- Isolated cases of neutropenia, thrombocytopenia and pancytopenia

(Gibbon, 2000:369; Hardman & Limbird, 1996:632).

1.6.3.2. Contra-indications

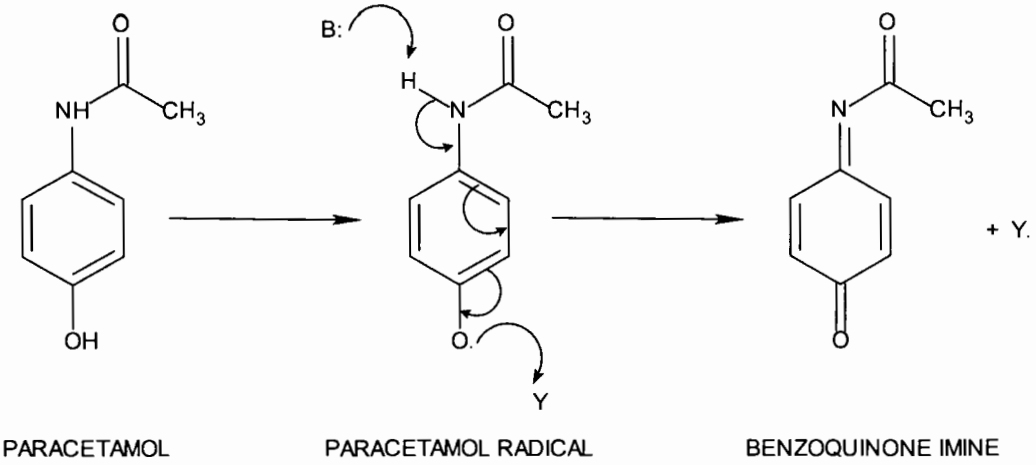
- Severe hepatic disease;
- Severe renal disease; and
- Use with caution in:
    - Alcoholism
    - Hepatic or renal dysfunction
    - Anaemia and haemopoietic disorders

(Gibbon, 2000:369).

1.6.4. TOXICITY

In adults, a small proportion of ingested paracetamol undergoes cytochrome P450-mediated *N*-hydroxylation to form a highly reactive intermediate called *N*-acetyl-benzoquinone imine. This intermediate normally reacts with sulphydryl groups in glutathione. When a very large dose of paracetamol is ingested, the metabolite is produced in quantities sufficient to deplete hepatic glutathione stores. When this happens, reaction of the metabolite with sulphydryl groups of hepatic proteins increases and this in turn may cause hepatic necrosis. It is thought that the necrosis is due to DNA fragmentation, caused by intracellular accumulation of Ca^{2+} and activation of Ca^{2+}-dependent endonuclease. Children have less capacity for glucuronidation than do adults and are at greater risk of suffering toxic effects. It is also known that ethanol induces cytochrome P450, thus increasing the production of *N*-acetyl-benzoquinone imine (Hardman & Limbird, 1996:632; Silverman, 1992:313).

1.6.4.1. Hepatic bioactivation of paracetamol



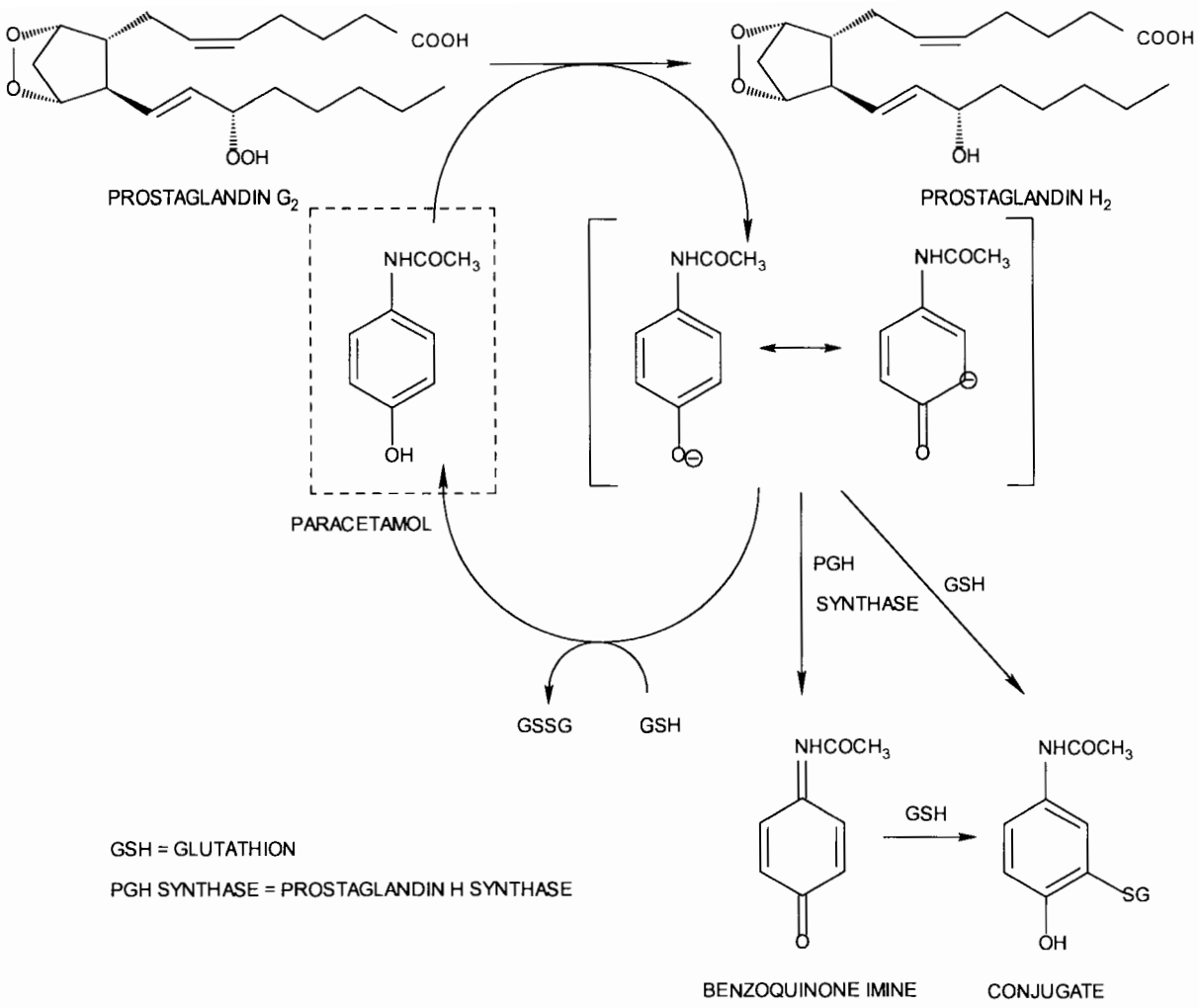
Scheme 3: Hepatic cytochrome P-450-dependent bioactivation of paracetamol

(Silverman, 1992:314)

This mechanism involves cytochrome P450-dependent paracetamol radical formation followed by second electron transfer to oxygen (scheme 3). The acceptor Y in scheme 3 may be O_2 or oxygen bound to the heme of cytochrome P450. The resulting electrophilic metabolite is responsible for the observed hepatotoxicity (Silverman, 1992:314).

1.6.4.2. Renal bioactivation of paracetamol

Paracetamol overdose may also cause renal damage in some cases. Cytochrome P-450 activity is low in the kidneys and it is probably not the principle enzyme responsible for paracetamol toxicity in this organ. Prostaglandin H synthase (also known as cyclo-oxygenase) is the enzyme that converts arachidonic acid to prostaglandins and is present in high concentrations in the kidneys. When cyclo-oxygenase catalyses the reduction of PGG_2 to PGH_2, it can simultaneously co-oxidise paracetamol to an intermediate that reacts with glutathione to form the glutathione conjugate (scheme 4). Again the electrophilic, and highly reactive, intermediate is responsible for the toxicity (Silverman, 1992:314).



Scheme 4: Renal prostaglandin H synthase dependent bioactivation of paracetamol

(Silverman, 1992:315)

1.6.4.3. Treatment of paracetamol toxicity

The most serious toxic effect of paracetamol is dose-dependent, potentially fatal hepatic necrosis. A single dose of 10-15 g may cause hepatotoxicity in adults, with doses of 20-25 g or more being potentially fatal. Initial symptoms following acute poisoning may include nausea, vomiting, anorexia and abdominal pain. Clinical manifestations of hepatic damage only become apparent after 2-4 days. The potential severity of hepatic necrosis can be predicted from the paracetamol plasma level (chart 1) and half-life ($T_{1/2}$) observed in the patient:

- $T_{1/2}$ > 4 hours: Necrosis is likely to occur

- $T_{1/2}$ > 12 hours: Hepatic coma is likely

(Hardman & Limbird, 1996:632; Katzung, 2001:615).

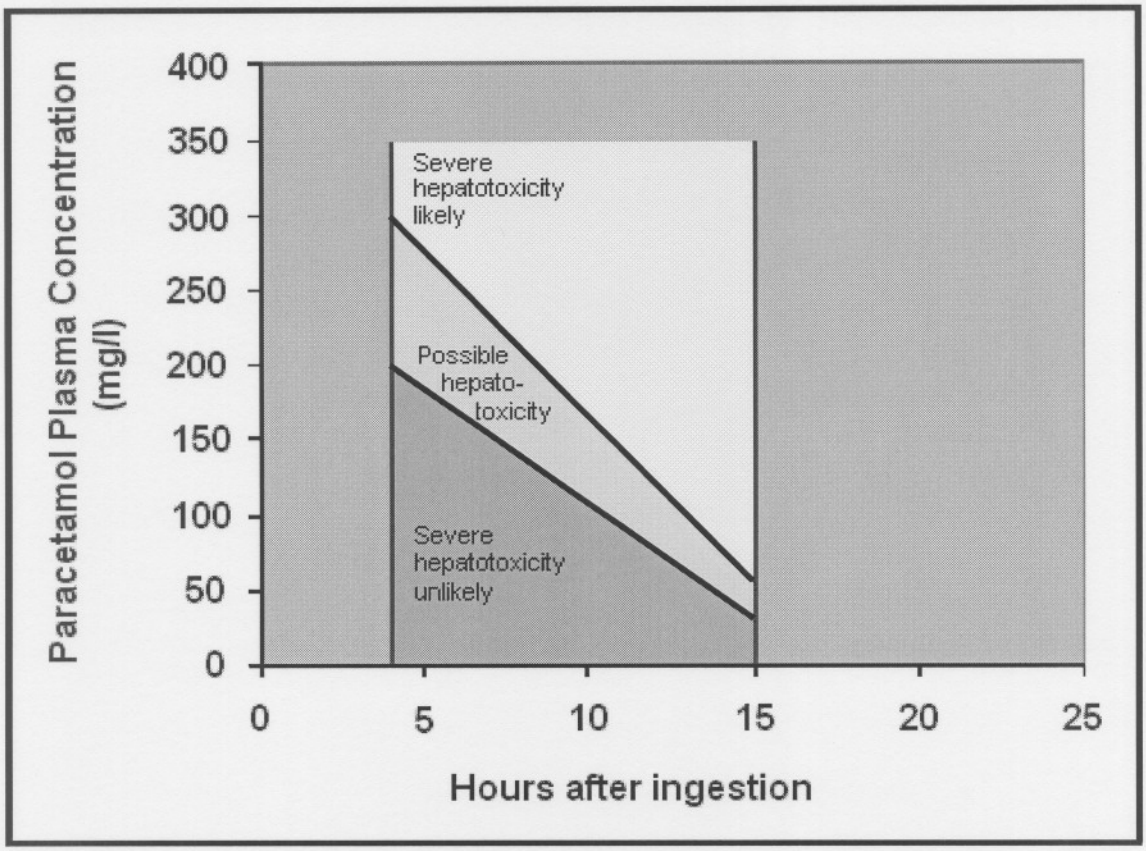


Chart 1: Paracetamol plasma concentration as measure of possible hepatotoxicity

(Sommers, 1997:38)

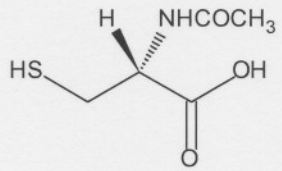


Figure 4: Acetylcysteine (Lund, 1994:710)

In all cases where significant poisoning is suspected, treatment should not be delayed while awaiting laboratory results. Gastric lavage should preferably be performed within 4 hours of ingestion. *N*-acetylcysteine (figure 4) is a useful antidote

if administered less than 36 hours after ingestion of paracetamol, although it is most effective if less than 10 hours have elapsed. This sulphydryl compound probably acts by replenishing hepatic glutathione stores and neutralising toxic metabolites (Hardman & Limbird, 1996:632; Katzung, 2001:615).

1.7. CONCLUSION

Paracetamol is currently the analgesic / antipyretic which is most commonly used. It is inexpensive, available without prescription and is relatively safe in therapeutic doses. Since toxic effects are so rare, paracetamol is the drug of choice for treating pain and fever in children. Its analgesic and antipyretic effects are comparable to that of aspirin, but paracetamol is safe in many instances where the use of aspirin may be contra-indicated: aspirin allergy; haemophilia; history of peptic ulcers; where bronchospasm is precipitated by aspirin, and viral conditions in children.

The poor water-solubility of paracetamol, as well as its bitter taste, complicates the formulation of oral paediatric preparations. Ethanol increases the risk of toxic effects and therefore its use is undesirable, despite it being capable of easily dissolving high concentrations of paracetamol. Consequently, alternative co-solvents are discussed in chapter 2.

CHAPTER 2

PREFORMULATION AND EARLY FORMULATION

2.1. INTRODUCTION

Preformulation was conducted as a literature study since paracetamol, and the formulation thereof into oral dosage forms, has been extensively described by many authors. The solubility, melting point and stability of paracetamol were some features of the drug that Wells & Aulton (1996:224) suggest should be investigated during the learning phase known as preformulation. Another important issue was establishing the toxicity and solubilisation characteristics of the co-solvents that were later used in formulation.

Early formulation was based on the knowledge gained during preformulation and was to some extent a process of trial-and-error.

2.2. GENERAL CONSIDERATIONS – ORAL SUSPENSIONS AND SOLUTIONS

Liquid oral dosage forms are easier to swallow than solid dosage forms such as tablets and capsules (Billany, 1996:254). This makes liquid dosage forms particularly useful in paediatrics, geriatrics and in cases where the ability to swallow has been compromised due to injury or disability.

Aqueous oral solutions and suspensions should be made with purified water which is prepared by deionisation or distillation of potable water (Collett, 1990:87).

2.2.1. ORAL SUSPENSIONS

Physical properties of a well-formulated suspension (Billany, 1996:269-270 & Collett, 1990:99):

- After shaking the container, the disperse phase should remain homogenously distributed throughout the dispersion medium for a sufficient period of time to allow for the removal of the required dose. This will ensure uniformity of dose.

- Mild agitation of the product should be all that is required to resuspend the sediment that is produced on storage.
- If the product is thickened to delay sedimentation, the viscosity should not be so high as to impede removal of the product from its container or measuring of the required dose.
- The suspended particles should be small and uniformly sized to ensure that the product has a smooth, pleasant and non-gritty texture.

2.2.2. ORAL SOLUTIONS

"A solution is a homogenous one-phase system consisting of two or more components. The solvent is that phase in which the dispersion occurs and the solute is that component which is dispersed as small molecules or ions in the solvent" – Billany (1996:254).

From this definition it is obvious that a solution is a clear liquid, free from particles or sediment.

2.3. SOLUBILISATION OF PARACETAMOL IN ORAL PAEDIATRIC SOLUTIONS

Billany (1996:256) describes the use of co-solvents to improve the solubility of poorly water-soluble drugs. The solubility of a drug is often greater in a mixed system than can be predicted from its solubility in each individual solvent. An ideal co-solvent would be a non-toxic, water-miscible vehicle in which the drug is highly soluble.

2.3.1. ETHANOL

Paracetamol is easily dissolved in ethanol (1 part in 7) and ethanol does not adversely affect patient acceptability.

The South African Medicines Control Council (MCC) allows the following maximum ethanol concentrations for medicines intended for oral ingestion (MCC, 2003:2):

- 0.5% (v/v) for children under 6 years of age;

- 5.0% (v/v) for children aged 6-12 years; and
- 10.0% (v/v) for persons over 12 years of age.

As liquid paracetamol dosage forms are mainly intended for use by young children, 0.5% (v/v) would be the appropriate limit.

Though it may seem an ideal solvent for paracetamol, ethanol increases the risk of hepatotoxicity (as discussed in section 1.6.4) when used with this drug. The use of ethanol should be minimised, or ideally avoided, in formulating products containing paracetamol. Alternative co-solvents are discussed in the following paragraphs.

2.3.2. GLYCEROL

The solubility of paracetamol in glycerol is 1 part in 40. Glycerol has a relatively low toxicity and its adverse effects are mainly due to its dehydrating action. Large doses (1-1.5 g/kg body weight) can be administered (Martindale, 1993:1374). The use of glycerol as co-solvent in formulating an oral paracetamol solution would probably be limited by factors other than toxicity. These may include patient acceptability and production cost.

2.3.3. POLYETHYLENE GLYCOLS (PEG's)

PEG's are condensation polymers of ethylene oxide and water. The name of each PEG is followed by a number indicating its approximate average molecular weight. The physical properties of these substances vary; PEG's with molecular weights between 200 and 600 are viscous liquids, while those with molecular weights greater than 1000 are solids. Toxicity of the PEG's is low and inversely proportional to molecular weight (Martindale, 1993:1384).

The estimated acceptable intake of PEG's is 10 mg/kg/day (WHO, 1980:18).

2.3.4. PROPYLENE GLYCOL

Propylene glycol is a clear, odourless, viscous, hygroscopic liquid with a characteristic taste. It is used extensively in pharmaceutical manufacturing as a solvent and vehicle (Martindale, 1993:1406). The solubility of paracetamol in propylene glycol is 1 part in 9.

The estimated acceptable intake of propylene glycol is 25 mg/kg/day (WHO, 1974:37).

2.3.5. POLYVINYLPYRROLIDONE (PVP)

PVP is a fine white, odourless, hygroscopic powder. It is widely used as a suspending and dispersing agent. It is also used for granulating, tablet binding and as a coating agent (Martindale, 1993:1220-1221).

The estimated acceptable intake of PVP is 50 mg/kg/day (BASF, 1993:239).

2.3.6. MEGLUMINE

Meglumine (figure 5) is a white, odourless powder which is highly soluble in water (1 part in 1). It is an organic base and is used for the preparation of organic acid salts (Martindale, 1993:1385).

De Villiers *et al.* (1999:967-972) investigated the influence of meglumine on the dissolution and complexing properties of ibuprofen and ketoprofen, when mixed with these drugs. Meglumine improved the solubility and dissolution of both drugs, but only ibuprofen showed evidence of possible complexation with meglumine. The increase in solubility could be the result of water-soluble salts formed between meglumine and the acidic drugs.

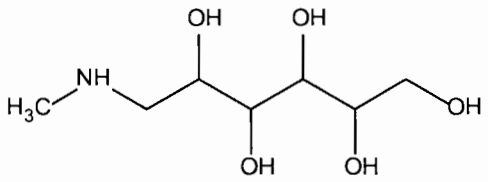


Figure 5: *N*-Methylglucamine (Meglumine) (De Villiers *et al.,* 1999:968).

The effect of meglumine on paracetamol solubility was briefly investigated and it did indeed improve water solubility markedly. However, the pH values of these solutions were so high (table 1) that meglumine is rendered totally useless as a co-solvent for paracetamol. Further investigations were abandoned.

Table 1: pH-values for paracetamol/meglumine solutions

MOLAR RATIO (MEGLUMINE : PARACETAMOL)	**pH**
1 : 1	9.80
0.9 : 1	9.75
0.8 : 1	9.70
0.7 : 1	9.63
0.6 : 1	9.56
0.5 : 1	9.49

2.4. PATIENT ACCEPTABILITY

Patient acceptability is especially important when formulating paediatric medicines. The product must not have an unpleasant appearance, taste or smell. Since paracetamol has a bitter taste, which is even more apparent in solution, it is necessary to sweeten the product. Artificial sweeteners were used in this study, because they don't cause tooth-decay and are safe for use in diabetes. Flavourings were selected that would appeal to children, namely bubblegum, strawberry and grape. Colorants were chosen to complement the flavours.

2.5. STABILITY

The stability of drugs in aqueous solution is often poorer than in their solid state, particularly if they are susceptible to hydrolysis. The hydrolysis of paracetamol is both acid-catalysed and base-catalysed (section 1.3.3.2) and is at a minimum within the pH range 5 to 6.

Care should be taken that the co-solvent systems used for paracetamol solutions sufficiently solubilise the drug to ensure no recrystallisation takes place during storage, even if the product is exposed to varying environmental temperatures.

Suspensions should not exhibit crystal-growth, as this will result in an unpleasant texture and poor patient acceptability.

Liquid dosage forms often provide suitable media for the growth of micro-organisms. This necessitates the inclusion of preservatives.

2.6. CONCLUSION

The physico-chemical properties of paracetamol, as well as its formulation into liquid dosage forms were studied during preformulation. It has been shown that ethanol increases the risk of paracetamol-induced hepatotoxicity and that its use should be avoided. Various co-solvents were considered as alternative to ethanol for use in aiding the solubility of paracetamol.

General methods for the formulation of liquid paracetamol dosage forms were developed during early formulation. The specific trial formulations are discussed in chapter 3.

CHAPTER 3

THE FORMULATION OF ORAL PAEDIATRIC PARACETAMOL PREPARATIONS

3.1. INTRODUCTION

During this study, various oral paediatric paracetamol preparations were formulated. These included suspensions, micro-fine suspensions and solutions. Products with low toxicity and good patient acceptability that showed satisfactory initial stability were selected to undergo further stability testing. Potential stability was determined by visual evaluation over a few days' storage at room temperature. A further consideration in the selection of the oral solutions, was the effect that 0.50% (v/v) ethanol might have on the stability of a product.

3.2. CHEMICALS USED

The chemicals used during formulation are given in table 1.

Table 1 : Chemicals used

Function	Chemical	Grade / Quality	Supplier
Co-solvent	Ethanol absolute	Chemically pure 99% min.	Saarchem
	Glycerol	Chemically pure 99% min.	Saarchem
	PEG 4000	Analytical reagent	Merck Chemicals
	Propylene glycol	Chemically pure	Saarchem
	PVP	Chemically pure	BASF
	Sorbitan tri-stearate	Chemically pure	Saarchem
Active	Paracetamol	99 - 101%	Fine Chemicals

Table 1 : Chemicals used (continued)

Preservative	Methyl paraben	99 - 101%	BDH Laboratory Reagents
	Propyl paraben	99 - 101%	BDH Laboratory Reagents
Carrier	Sorbitol solution 70% B.P.	B.P. specifications	Brunel Laboratoria
	Syrup B.P.	B.P. specifications	Brunel Laboratoria
Colour	Blackcurrent	Industrial	H & R
	Brilliant blue	Industrial	BASF
	Raspberry red H1277	Industrial	Adcock Ingram
	Yellow	Industrial	Pointing
Flavour	Bubblegum LR.5490	Industrial	H & R
	Grape 52.132C	Industrial	Firmenich
	Musk-mint TS 90622	Industrial	H & R
	Passionfruit LR 2.82	Industrial	H & R
	Raspberry 502.700C	Industrial	Firmenich
	Strawberry 502.301C	Industrial	Firmenich
	Spearmint LR 34486	Industrial	H & R
Base	Sodium hydroxide pellets	Analysed reagent 98% min.	Saarchem
Sweetener	Sodium saccharin	99.0 - 101.0%	Merck Chemicals
Taste enhancer	Citric acid	USP specifications	Roche
	Sodium chloride	Chemically pure 99% min.	Saarchem
Hydrocolloid	Hydroxyethylcellulose	For experimental use	Aqualon
	Xanthan gum	USP specifications	Tranarc
Solvent	Water	Purified	Millipore water purification system

3.3. FORMULAE

3.3.1. SUSPENSIONS

One suspension was formulated. This product was selected for further stability testing, because it showed good initial stability and patient acceptability.

3.3.1.1. Formula 1

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	5.00 ml
PEG 4000	Co-solvent	-
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	-
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Raspberry red	Colour	0.01 g
Strawberry	Flavour	0.20 g
Sodium saccharin	Sweetener	0.10 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	0.50 g
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol and propylene glycol were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The paracetamol was suspended in 20 ml of purified water – suspension 1.
- Solution 1 and suspension 1 were mixed together, and the sorbitol solution 70% B.P. and another 20 ml of purified water was added – suspension 2.
- The sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 2.
- Suspension 2 and solution 2 were mixed together – suspension 3.
- The xanthan gum was mixed with the glycerol – mixture 1.
- Mixture 1 was added to suspension 3 and stirred until a uniform consistency was obtained.
- Purified water was added to volume (100 ml).

Results:

- A suspension with uniform particle distribution was obtained.
- Settling occurred upon standing, but the particles were easily re-suspended by shaking.
- This product underwent stability testing (see chapter 4).

3.3.2. MICRO-FINE SUSPENSIONS

Two micro-fine suspensions were formulated. Formula 1 was selected for further stability testing, because it showed good initial stability and patient acceptability.

3.3.2.1. Formula 1

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	5.00 ml
PEG 4000	Co-solvent	-
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	-
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Raspberry red H1277	Colour	0.01 g
Strawberry	Flavour	0.20 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Acid, taste enhancer	1.02 g
Sodium hydroxide	Base	0.635 g
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	0.50 g
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol and propylene glycol were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The sodium hydroxide was dissolved in 20 ml of purified water – solution 2.
- The paracetamol was dissolved in solution 2 by stirring – solution 3.
- The citric acid was dissolved in 20 ml of purified water – solution 4.
- Solution 4 was added to solution 3 and a fine precipitate of paracetamol was obtained – suspension 1.
- Solution 1 was mixed with suspension 1 and the sorbitol solution 70% B.P. was added – suspension 2.
- The sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solution 5 was mixed with suspension 2 – suspension 3.
- The xanthan gum was mixed with the glycerol – mixture 1.
- Mixture 1 was added to suspension 3 and stirred until a uniform consistency was obtained.
- Purified water was added to volume (100 ml).

Results:

- A fine suspension with uniform particle distribution was obtained.
- Settling occurred upon standing, but the particles were easily re-suspended by shaking.
- This product underwent stability testing (see chapter 4).

3.3.2.2. Formula 2

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	5.00 ml
PEG 4000	Co-solvent	-
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	-
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	20.00 ml
Syrup B.P.	Carrier	20.00 ml
Raspberry red H1277	Colour	0.01 g
Strawberry	Flavour	0.20 g
Sodium saccharin	Sweetener	0.50 g
Citric acid	Acid, taste enhancer	1.05 g
Sodium hydroxide	Base	0.635 g
Sodium chloride	Taste enhancer	0.50 g
Xanthan gum	Hydrocolloid	0.50 g
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol and propylene glycol were used as co-solvents in this formula.

- This formula was identical to formula 1, except for the addition of syrup B.P., sodium chloride and more citric acid. The amount of sodium saccharin was also decreased, because syrup B.P. was used.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The sodium hydroxide was dissolved in 20 ml of purified water – solution 2.
- The paracetamol was dissolved in solution 2 by stirring – solution 3.
- The citric acid was dissolved in 20 ml of purified water – solution 4.
- Solution 4 was added to solution 3 and a fine precipitate of paracetamol was obtained – suspension 1.
- Solution 1 was mixed with suspension 1 and the sorbitol solution 70% B.P. and syrup B.P. were added – suspension 2.
- The sodium saccharin, sodium chloride, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solution 5 was mixed with suspension 2 – suspension 3.
- The xanthan gum was mixed with the glycerol – mixture 1.
- Mixture 1 was added to suspension 3 and stirred until a uniform consistency was obtained.
- Purified water was added to volume (100 ml).

Results:

- A fine suspension with uniform particle distribution was obtained.
- Settling occurred upon standing, but the particles were easily re-suspended by shaking.
- No discernible difference in the product's initial stability occurred as a result of the changes to the formula. However, patient acceptability decreased, because the bitter taste of the paracetamol was more noticeable.

3.3.3. SOLUTIONS

Twenty-one solutions were formulated. Of these only formulae 18, 19, 20 and 21 underwent stability testing, since they were the most promising products regarding low toxicity, initial stability and patient acceptability.

3.3.3.1. Formula 1

Ingredient	**Function**	**Amount (g or ml / 100 ml)**
Ethanol	Co-solvent	-
Glycerol	Co-solvent	2.50 ml
PEG 4000	Co-solvent	10.00 g
Propylene glycol	Co-solvent	10.00 ml
PVP	Co-solvent	-
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.02 g
Sorbitol solution 70% B.P.	Carrier	30.00 ml
Syrup B.P.	Carrier	10.00 ml
-	Colour	-
-	Flavour	-
Sodium saccharin	Sweetener	0.10 g
Citric acid	Taste enhancer	0.03 g
Sodium chloride	Taste enhancer	0.50 g
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol, PEG 4000 and propylene glycol were used as co-solvents in this formula.
- PEG 4000 and propylene glycol concentrations used exceed recommended limits, but this product was only formulated for the purpose of developing a general practical method for the production of further oral solutions.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PEG 4000 was dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was stirred until the paracetamol had dissolved – solution 4.
- The sodium chloride, citric acid and sodium saccharin were added to solution 4 and the mixture was stirred until all additives had dissolved – solution 5.
- Sorbitol solution 70% B.P. and syrup B.P. were added and mixed into solution 5.
- Purified water was added to volume (100 ml).

Results:

- The paracetamol was easily dissolved and the solution was clear. This solution seemed stable, since no recrystallisation was observed after a few days' storage at room temperature.
- No further development of this formula was attempted, due to possible adverse effects that may result from ingesting potentially excessive amounts of PEG 4000 and propylene glycol.

3.3.3.2. Formula 2

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	20.00 ml
PEG 4000	Co-solvent	-
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Raspberry red H1277	Colour	0.008 g
Raspberry liquid	Flavour	0.01 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol, propylene glycol and PVP were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PVP was dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin was added to the sorbitol solution 70% B.P. and 5 ml of purified water and was stirred until the sodium saccharin had dissolved – solution 5.
- The colour and flavour were dissolved in 5 ml of purified water – solution 6.
- Solutions 4, 5 and 6 were mixed together.
- Purified water was added to volume (100 ml).

Results:

- The paracetamol did not dissolve completely with stirring and heating to 60°C.
- Only with stirring and heating to 75°C did the paracetamol eventually dissolve.
- The resulting solution was unstable, since minor recrystallisation was observed after only a few days' storage at room temperature.
- No further development of this formula was attempted, due to product instability.

3.3.3.3. Formula 3

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	30.00 ml
PEG 4000	Co-solvent	-
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Blackcurrant	Colour	0.008 g
Grape liquid	Flavour	0.01 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- This formula was identical to formula 2, except for an additional 10 ml of glycerol, which was added in an attempt to improve solubility.
- Glycerol, propylene glycol and PVP were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PVP was dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin was added to the sorbitol solution 70% B.P. and 5 ml of purified water and was stirred until the sodium saccharin had dissolved – solution 5.
- The colour and flavour were dissolved in 5 ml of purified water – solution 6.
- Solutions 4, 5 and 6 were mixed together.
- Purified water was added to volume (100 ml).

Results:

- With stirring and heating to 60°C, the paracetamol dissolved completely after about 60 to 90 minutes.
- The resulting solution appeared to be stable, since no recrystallisation was observed.
- No further development of this formula was attempted, due to the impractical method in terms of the long preparation time.

3.3.3.4. Formula 4

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	20.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	-
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.02 g
Sorbitol solution 70% B.P.	Carrier	20.00 ml
Syrup B.P.	Carrier	-
Yellow	Colour	0.01 g
Passionfruit	Flavour	0.10 g
Musk-mint	Flavour	0.10 g
Sodium saccharin	Sweetener	0.50 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol, PEG 4000 and propylene glycol were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PEG 4000 was dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, colour and flavour were dissolved in 5 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. and the syrup B.P. were added.
- Purified water was added to volume (100 ml).

Results:

- The paracetamol did not dissolve completely with stirring and heating to 60°C.
- Only with stirring and heating to 75°C did the paracetamol eventually dissolve.
- The resulting solution was unstable, since minor recrystallisation was observed after only a few days' storage at room temperature.
- No further development of this formula was attempted, due to product instability.

3.3.3.5. Formula 5

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	30.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	-
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.02 g
Sorbitol solution 70% B.P.	Carrier	20.00 ml
Syrup B.P.	Carrier	-
Yellow	Colour	0.01 g
Passionfruit	Flavour	0.10 g
Musk-mint	Flavour	0.10 g
Sodium saccharin	Sweetener	0.50 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- This formula was identical to formula 4, except for an additional 10 ml of glycerol, which was added in an attempt to improve solubility.

- Glycerol, PEG 4000 and propylene glycol were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PEG 4000 was dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, colour and flavour were dissolved in 5 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. and the syrup B.P. were added.
- Purified water was added to volume (100 ml).

Results:

- The paracetamol did not dissolve completely with stirring and heating to 60°C.
- Only with stirring and heating to 75°C did the paracetamol eventually dissolve.
- The resulting solution was unstable and slight recrystallisation was observed after only a few days' storage at room temperature.
- No further development of this formula was attempted, due to product instability.

3.3.3.6. Formula 6

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	20.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	1.00 g
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.02 g
Sorbitol solution 70% B.P.	Carrier	20.00 ml
Syrup B.P.	Carrier	20.00 ml
Brilliant blue	Colour	0.01 g
Bubblegum	Flavour	0.20 g
Sodium saccharin	Sweetener	0.50 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	0.50 g
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol, PEG 4000, propylene glycol and PVP were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PEG 4000 and PVP were dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, sodium chloride, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. and the syrup B.P. were added.
- Purified water was added to volume (100 ml).

Results:

- The paracetamol did not dissolve completely with stirring and heating to 60°C.
- Only with stirring and heating to 75°C did the paracetamol eventually dissolve.
- When solutions 4 and 5 were mixed together, the product turned cloudy.
- The resulting solution was unstable and slight recrystallisation was observed after only a few days' storage at room temperature.
- No further development of this formula was attempted, due to product instability and unsatisfactory appearance.

3.3.3.7. Formula 7

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	20.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.02 g
Sorbitol solution 70% B.P.	Carrier	20.00 ml
Syrup B.P.	Carrier	20.00 ml
Brilliant blue	Colour	0.01 g
Bubblegum	Flavour	0.20 g
Spearmint	Masks bitterness	1 drop
Sodium saccharin	Sweetener	0.50 g
Citric acid	Taste enhancer	0.03 g
Sodium chloride	Taste enhancer	0.50 g
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- This formula was identical to formula 6, except for an additional 2.33 g of PVP, which was added in an attempt to improve solubility.

- Glycerol, PEG 4000, propylene glycol and PVP were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PEG 4000 and PVP were dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, sodium chloride, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. and the syrup B.P. were added.
- Purified water was added to volume (100 ml).

Results:

- When solutions 4 and 5 were mixed together, the product turned cloudy.
- No further development of this formula was attempted, due to the unsatisfactory appearance of the product.

3.3.3.8. Formula 8

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	30.00 ml
PEG 4000	Co-solvent	-
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	-
Sorbitan tri-stearate	Co-solvent	0.92 g
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.02 g
Sorbitol solution 70% B.P.	Carrier	20.00 ml
Syrup B.P.	Carrier	20.00 ml
Brilliant blue	Colour	0.01 g
Bubblegum	Flavour	0.20 g
Sodium saccharin	Sweetener	0.50 g
Citric acid	Taste enhancer	0.03 g
Sodium chloride	Taste enhancer	0.50 g
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol, propylene glycol and sorbitan tri-stearate were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- Glycerol and sorbitan tri-stearate were added to solution 1 – solution 2.
- The paracetamol was added to solution 2 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 3.
- The sodium saccharin, sodium chloride, citric acid, colour and flavour were dissolved in 10 ml of purified water – solution 4.
- Solutions 3 and 4 were mixed together and the sorbitol solution 70% B.P. and the syrup B.P. were added.
- Purified water was added to volume (100 ml).

Results:

- The paracetamol did not dissolve completely.
- Sorbitan tri-stearate gave the product an unpleasant after-taste and caused foaming when the product was stirred or shaken.
- No further development of this formula was attempted, due to the poor solubilisation properties of the co-solvent system, as well as poor patient acceptability.

3.3.3.9. Formula 9

Ingredient	Function	Amount
Ethanol	Co-solvent	-
Glycerol	Co-solvent	20.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	1.00 g
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	20.00 ml
Syrup B.P.	Carrier	-
Brilliant blue	Colour	0.01 g
Bubblegum	Flavour	0.20 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	0.50 g
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- This formula was similar to formula 7. The co-solvent system used was identical, but the colour, flavour and carrier differed. Unlike formula 7, formula 9 contained no syrup B.P. and was therefore sugar-free.
- Glycerol, PEG 4000, propylene glycol and PVP were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PEG 4000 and PVP were dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. was added.
- Purified water was added to volume (100 ml).

Results:

- With stirring and heating to 60°C, the paracetamol dissolved completely.
- The resulting solution was unstable and minor recrystallisation was observed after only a few days' storage at room temperature.
- No further development of this formula, due to product instability.
- Despite no special effort to mask the bitter taste of paracetamol, the product showed better initial stability and patient acceptability than formula 7.

3.3.3.10. Formula 10

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	20.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Blackcurrant	Colour	0.008 g
Grape	Flavour	0.20 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- This formula was identical to formula 9, except for an additional 2.33 g of PVP, which was added in an attempt to improve solubility.
- Glycerol, PEG 4000, propylene glycol and PVP were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PEG 4000 and PVP were dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. was added.
- Purified water was added to volume (100 ml).

Results:

- With stirring and heating to 60°C, the paracetamol dissolved completely.
- The resulting solution appeared to be stable and no recrystallisation was observed.
- Although the product showed acceptable initial stability, it was decided to limit the study to four solutions. Its stability testing was rejected in favour of solutions 18, 19, 20 and 21.

3.3.3.11. Formula 11

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	20.00 ml
PEG 4000	Co-solvent	-
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	0.92 g
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Blackcurrant	Colour	0.008 g
Grape	Flavour	0.20 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol, propylene glycol, PVP and sorbitan tri-stearate were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PVP and sorbitan tri-strearate were dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. was added.
- Purified water was added to volume (100 ml).

Results:

- With stirring and heating to 60°C, the paracetamol dissolved completely.
- The resulting solution was unstable and slight recrystallisation was observed after only a few days' storage at room temperature.
- Sorbitan tri-stearate caused foaming when the product was stirred or shaken, as well as an unpleasant after-taste.
- No further development of this formula was attempted, due to product instability.

3.3.3.12. Formula 12

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	30.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	-
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Blackcurrant	Colour	0.008 g
Grape	Flavour	0.20 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol, PEG 4000, and PVP were used as co-solvents in this formula.

Method:

- The PEG 4000 and PVP were dissolved in 20 ml of purified water – solution 1.
- Glycerol was added to solution 1 – solution 2.
- The parabens were dissolved in solution 2 by stirring and heating to 60°C – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. was added.
- Purified water was added to volume (100 ml).

Results:

- The parabens required a considerable amount of time to fully dissolve in solution 2.
- With stirring and heating to 60°C, the paracetamol dissolved completely.
- No further development of this formula was attempted, due to the impracticality of this method.

3.3.3.13. Formula 13

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	30.00 ml
PEG 4000	Co-solvent	-
Propylene glycol	Co-solvent	-
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	0.92 g
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Blackcurrant	Colour	0.008 g
Grape	Flavour	0.20 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol, PVP and sorbitan tri-stearate were used as co-solvents in this formula.

Method:

- The PVP and sorbitan tri-stearate were dissolved in 20 ml of purified water – solution 1.
- Glycerol was added to solution 1 – solution 2.
- The parabens were dissolved in solution 2 by stirring and heating to 60°C – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. was added.
- Purified water was added to volume (100 ml).

Results:

- The parabens required a considerable amount of time to fully dissolve in solution 2.
- With stirring and heating to 60°C, the paracetamol dissolved completely.
- Sorbitan tri-stearate caused foaming when the product was stirred or shaken, as well as an unpleasant after-taste.
- The resulting solution was unstable and slight recrystallisation was observed after only a few days' storage at room temperature.
- No further development of this formula was attempted, due to the impracticality of this method and product instability.

3.3.3.14. Formula 14

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	20.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	0.92 g
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Blackcurrant	Colour	0.008 g
Grape	Flavour	0.20 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol, PEG 4000, propylene glycol, PVP and sorbitan tri-stearate were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PVP, PEG 4000 and sorbitan tri-stearate were dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. was added.
- Purified water was added to volume (100 ml).

Results:

- The paracetamol dissolved completely with stirring and heating to 60°C.
- The resulting solution appeared to be stable and no recrystallisation was observed.
- Sorbitan tri-stearate caused foaming when the product was stirred or shaken, as well as an unpleasant after-taste.
- No further development of this formula was attempted, due to poor patient acceptability.

3.3.3.15. Formula 15

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	30.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	-
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	0.92 g
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Blackcurrant	Colour	0.008 g
Grape	Flavour	0.20 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol, PEG 4000, PVP and sorbitan tri-stearate were used as co-solvents in this formula.

Method:

- The PEG 4000, PVP and sorbitan tri-stearate were dissolved in 20 ml of purified water – solution 1.
- Glycerol was added to solution 1 – solution 2.
- The parabens were dissolved in solution 2 by stirring and heating to 60°C – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. was added.
- Purified water was added to volume (100 ml).

Results:

- The parabens required a considerable amount of time to fully dissolve in solution 2.
- With stirring and heating to 60°C, the paracetamol dissolved completely.
- Sorbitan tri-stearate gave the product an unpleasant after-taste and caused it to foam when stirred or shaken.
- No further development of this formula was attempted, due to the impracticality of this method and poor patient acceptability.

3.3.3.16. Formula 16

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	30.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Blackcurrant	Colour	0.01 g
Grape	Flavour	0.20 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol, PEG 4000, propylene glycol and PVP were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PVP and PEG 4000 were dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. was added.
- Purified water was added to volume (100 ml).

Results:

- The paracetamol dissolved completely with stirring and heating to 60°C.
- The resulting solution appeared to be stable and no recrystallisation was observed.
- Further development: Patient acceptability was improved by increasing the viscosity of the product (see formulae 17 – 21).

3.3.3.17. Formula 17

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	30.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Blackcurrant	Colour	0.01 g
Grape	Flavour	0.20 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	-
Hydroxyethylcellulose	Hydrocolloid	0.50 g
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol, PEG 4000, propylene glycol and PVP were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PVP and PEG 4000 were dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the 20 ml of glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. was added – solution 6.
- The hydroxyethylcellulose was mixed with 10 ml of glycerol – mixture 1.
- Mixture 1 was added to solution 6 and stirred until a uniform consistency was obtained.
- Purified water was added to volume (100 ml).

Results:

- The paracetamol dissolved completely with stirring and heating to 60°C.
- The hydroxyethylcellulose used in this instance, was suspected to have been exposed to excess moisture during storage. Its gelling properties were reduced and it was nearly impossible to attain a uniform consistency.
- Xanthan gum was used as hydrocolloid instead in the following formulae.

3.3.3.18. Formula 18

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	30.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Raspberry red	Colour	0.01 g
Strawberry	Flavour	0.20 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	0.50 g
Hydroxyethylcellulose	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- Glycerol, PEG 4000, propylene glycol and PVP were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PVP and PEG 4000 were dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the 20 ml of glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. was added – solution 6.
- The xanthan gum was mixed with 10.00 ml of glycerol – mixture 1.
- Mixture 1 was added to solution 6 and stirred until a uniform consistency was obtained.
- Purified water was added to volume (100 ml).

Results:

- The paracetamol dissolved completely with stirring and heating to 60°C.
- The resulting solution appeared to be stable and no recrystallisation was observed.
- This product underwent stability testing (see chapter 4).

3.3.3.19. Formula 19

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	-
Glycerol	Co-solvent	30.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	1.20 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Raspberry red	Colour	0.01 g
Strawberry	Flavour	0.20 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	0.50 g
Hydroxyethylcellulose	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- This formula was identical to formula 18, except for the paracetamol content, which was halved in this formula, in an attempt to determine whether the paracetamol concentration would affect the stability of the product.

- Glycerol, PEG 4000, propylene glycol and PVP were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PVP and PEG 4000 were dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the 20 ml of glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. was added – solution 6.
- The xanthan gum was mixed with 10 ml of glycerol – mixture 1.
- Mixture 1 was added to solution 6 and stirred until a uniform consistency was obtained.
- Purified water was added to volume (100 ml).

Results:

- The paracetamol dissolved completely with stirring and heating to 60°C.
- The resulting solution appeared to be stable and no recrystallisation was observed.
- This product underwent stability testing (see chapter 4).

3.3.3.20. Formula 20

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	0.50 ml
Glycerol	Co-solvent	30.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	2.40 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Raspberry red	Colour	0.01 g
Strawberry	Flavour	0.20 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	0.50 g
Hydroxyethylcellulose	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- This formula was identical to formula 18, except for the addition of 0.50 ml of ethanol, as allowed by the Medicines Control Council (MCC), in order to determine its effect on the stability of the product.

- Glycerol, PEG 4000, propylene glycol, ethanol and PVP were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PVP and PEG 4000 were dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the 20 ml of glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The ethanol, sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. was added – solution 6.
- The xanthan gum was mixed with 10 ml of glycerol – mixture 1.
- Mixture 1 was added to solution 6 and stirred until a uniform consistency was obtained.
- Purified water was added to volume (100 ml).

Results:

- The paracetamol dissolved completely with stirring and heating to 60°C.
- The resulting solution appeared to be stable and no recrystallisation was observed.
- This product underwent stability testing (see chapter 4).

3.3.3.21. Formula 21

Ingredient	Function	Amount (g or ml / 100 ml)
Ethanol	Co-solvent	0.50 ml
Glycerol	Co-solvent	30.00 ml
PEG 4000	Co-solvent	0.50 g
Propylene glycol	Co-solvent	1.67 g
PVP	Co-solvent	3.33 g
Sorbitan tri-stearate	Co-solvent	-
Paracetamol	Active	1.20 g
Methyl paraben	Preservative	0.10 g
Propyl paraben	Preservative	0.01 g
Sorbitol solution 70% B.P.	Carrier	17.00 ml
Syrup B.P.	Carrier	-
Raspberry red	Colour	0.01 g
Strawberry	Flavour	0.20 g
Sodium saccharin	Sweetener	0.75 g
Citric acid	Taste enhancer	-
Sodium chloride	Taste enhancer	-
Xanthan gum	Hydrocolloid	0.50 g
Hydroxyethylcellulose	Hydrocolloid	-
Purified water	Solvent	To volume (100 ml)

Special notes:

- This formula was identical to formula 19, except for the addition of 0.50 ml of ethanol, as allowed by the MCC, in order to determine its effect on the stability

of the product.

- Glycerol, PEG 4000, propylene glycol, ethanol and PVP were used as co-solvents in this formula.

Method:

- The parabens were dissolved in the propylene glycol – solution 1.
- The PVP and PEG 4000 were dissolved in 20 ml of purified water – solution 2.
- Solutions 1 and 2 were mixed and the 20 ml of glycerol was added – solution 3.
- The paracetamol was added to solution 3 and the resulting mixture was heated to 60°C, stirred until the paracetamol had dissolved, and allowed to cool to room temperature – solution 4.
- The ethanol, sodium saccharin, colour and flavour were dissolved in 10 ml of purified water – solution 5.
- Solutions 4 and 5 were mixed together and the sorbitol solution 70% B.P. was added – solution 6.
- The xanthan gum was mixed with 10 ml of glycerol – mixture 1.
- Mixture 1 was added to solution 6 and stirred until a uniform consistency was obtained.
- Purified water was added to volume (100 ml).

Results:

- The paracetamol dissolved completely with stirring and heating to 60°C.
- The resulting solution appeared to be stable and no recrystallisation was observed.
- This product underwent stability testing (see chapter 4).

3.4. SIMPLIFIED METHOD FOR DETERMINING THE MAXIMUM CONCENTRATION OF ANY EXCIPIENT

The recommended acceptable daily intake (mg/kg/day) of each co-solvent used in this study is given in chapter 2. Maximum daily dose is generally given as a mass, but with liquid dosage forms this has to be converted to concentration, taking into account the volume administered. The maximum concentration of co-solvent to be used in the formulae discussed in this chapter was obtained with calculations based on a child of 10 kg receiving three 5 ml doses per day.

Though the calculations used in this study are convenient and simple, they have severe shortcomings – when these preparations are administered four times daily, or if a larger child requires a dose of 10 ml, the patient may inadvertently receive an overdose of the excipient. Therefore, an equation integrating all the variables determining excipient concentration was derived and reduced to a simple equation which is universally applicable.

$$\frac{Concentration\ excipient}{Concentration\ active\ ingredient} = \frac{Acceptable\ daily\ excipient\ intake}{Acceptable\ daily\ active\ ingredient\ intake}$$

This equation can be used if the acceptable daily intake of both the excipient and the active ingredient is known. A ratio is obtained from which the excipient concentration can be deduced once a convenient concentration for the active ingredient has been chosen. The amount of excipient ingested is determined by the volume taken and is directly proportional to the excipient concentration and inversely proportional to the concentration of the active ingredient.

In this study the paracetamol concentration, the acceptable paracetamol intake and the acceptable co-solvent intake should have determined the concentration of co-solvent not to be exceeded in a formula:

$$\frac{Concentration\ PEG}{Concentration\ paracetamol} = \frac{Acceptable\ daily\ PEG\ intake}{Acceptable\ daily\ paracetamol\ intake}$$

$$\frac{Concentration\ PEG}{2.4\,g/100\,ml} = \frac{10\,mg/kg/day}{40\,mg/kg/day}$$

$$Concentration\ PEG = 0.6\,g/100\,ml$$

$$\frac{Concentration\ propylene\ glycol}{Concentration\ paracetamol} = \frac{Acceptable\ daily\ propylene\ glycol\ intake}{Acceptable\ daily\ paracetamol\ intake}$$

$$\frac{Concentration\ propylene\ glycol}{2.4\,g/100\,ml} = \frac{25\,mg/kg/day}{40\,mg/kg/day}$$

$$Concentration\ propylene\ glycol = 1.5\,g/100\,ml$$

$$\frac{Concentration\ PVP}{Concentration\ paracetamol} = \frac{Acceptable\ daily\ PVP\ intake}{Acceptable\ daily\ paracetamol\ intake}$$

$$\frac{Concentration\ PVP}{2.4\,g/100\,ml} = \frac{50\,mg/kg/day}{40\,mg/kg/day}$$

$$Concentration\ PVP = 3.0\,g/100\,ml$$

3.5. CONCLUSION

In total, twenty-four oral paediatric paracetamol preparations were formulated. Six of these were selected to undergo stability testing. Selection was based on appearance, potential stability, as observed over a few days' storage at room temperature, and patient acceptability.

Those formulations that were selected for stability trials included:

- Suspensions
    - Formula 1.
- Micro-fine suspensions
    - Formula 1.
- Solutions
    - Formula 18: Containing 2.40 g / 100 ml paracetamol and no ethanol;
    - Formula 19: Containing 1.20 g / 100 ml paracetamol and no ethanol;
    - Formula 20: Containing 2.40 g / 100 ml paracetamol and 0.50 % v/v ethanol; and
    - Formula 21: Containing 1.20 g / 100 ml paracetamol and 0.50 % v/v ethanol.

These four solutions all contained the same co-solvent systems and differed only in their paracetamol and ethanol contents. This was done in order to determine, by comparison during stability testing (as discussed in chapter 4), the possible effects that 0.50 % v/v of ethanol and varying paracetamol concentrations would have on the stability of these solutions.

CHAPTER 4

STABILITY TESTING

4.1. INTRODUCTION

"The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions." – ICH, 2003:1.

"Stability studies should include testing of those attributes of the drug product that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy. The testing should cover, as appropriate, the physical, chemical, biological, and microbiological attributes, preservative content (e.g. antioxidant, antimicrobial preservative), and functionality tests (e.g. for a dose delivery system)." – ICH, 2003:6.

4.2. STABILITY PROGRAM

All products were tested under ICH conditions (ICH, 2003:4) over a period of three months at three different storage temperatures. Stability tests applicable to each formulation were conducted.

4.2.1. STORAGE TEMPERATURES

Sample batches of the six products formulated were stored at three different temperatures to determine their physical and chemical stability. The samples were stored in controlled climatic chambers under the following conditions:

- 5°C ± 3°C;
- 25°C ± 2°C / 60% RH ± 5%RH;
- 40°C ± 2°C / 75% RH ± 5%RH.

(ICH, 2003:9).

4.2.2. STABILITY TESTS CONDUCTED

Appropriate tests, as required by the South African Medicines Control Council (MCC, 2003:21-23) for stability studies, were conducted on the samples at four test intervals (tables 1 and 2). Calibrated and/or validated apparatus was used to conduct all tests.

Table 1 : Stability tests conducted on the solutions

STABILITY TESTS									
TEST	INITIAL	1 MONTH			2 MONTHS			3 MONTHS	
		5°C	25°C / 60% RH	40°C / 75% RH	5°C	25°C / 60% RH	40°C / 75% RH	5°C	25°C / 60% RH	40°C / 75% RH
APPEARANCE	✓	✓	✓	✓	×	✓	✓	×	✓	✓
pH	✓	✓	✓	✓	×	✓	✓	×	✓	✓
RELATIVE DENSITY	✓	✓	✓	✓	×	✓	✓	×	✓	✓
VISCOSITY	✓	✓	✓	✓	×	✓	✓	×	✓	✓
PRESERVATIVE EFFICACY	✓	×	×	×	×	×	×	×	×	✓
PARACETAMOL ASSAY	✓	✓	✓	✓	×	✓	✓	×	✓	✓
DEGRADATION PRODUCT 4-AMINOPHENOL	✓	✓	✓	✓	×	✓	✓	×	✓	✓
PRESERVATIVE ASSAY	✓	✓	✓	✓	×	✓	✓	×	✓	✓

Table 2 : Stability tests conducted on the suspensions

STABILITY TESTS										
TEST	INITIAL	1 MONTH			2 MONTHS			3 MONTHS		
		5°C	25°C / 60% RH	40°C / 75% RH	5°C	25°C / 60% RH	40°C / 75% RH	5°C	25°C / 60% RH	40°C / 75% RH
APPEARANCE	✓	✓	✓	✓	×	✓	✓	×	✓	✓
pH	✓	✓	✓	✓	×	✓	✓	×	✓	✓
RELATIVE DENSITY	✓	✓	✓	✓	×	✓	✓	×	✓	✓
PARTICLE SIZE	✓	✓	✓	✓	×	✓	✓	×	✓	✓
VISCOSITY	✓	✓	✓	✓	×	✓	✓	×	✓	✓
PRESERVATIVE EFFICACY	✓	×	×	×	×	×	×	×	×	✓
PARACETAMOL ASSAY	✓	✓	✓	✓	×	✓	✓	×	✓	✓
DEGRADATION PRODUCT 4-AMINOPHENOL	✓	✓	✓	✓	×	✓	✓	×	✓	✓
HPLC PRESERVATIVE ASSAY	✓	✓	✓	✓	×	✓	✓	×	✓	✓

4.3. TEST METHODS AND RESULTS

All tests were conducted under Good Laboratory Practice (GLP) conditions. Appearance, pH, relative density and viscosity assessment was done according to standard in-house operating procedures based on internationally accepted quality guidelines.

4.3.1. APPEARANCE

Results from the monthly assessment of the colour, flavour and texture of samples are given in tables 3 and 4.

Table 3 : The appearance of the solutions over three months of stability testing

APPEARANCE					
PRODUCT	**INITIAL**	**STORAGE TEMPERATURE**	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
SOLUTION : 2.4% PARACETAMOL (ALCOHOL FREE)	Strawberry-flavoured clear, pink solution with no apparent crystallisation.	5°C	No change	-	-
		25°C / 60% RH	No change	No change	No change
		40°C / 75% RH	No change	No change	No change
SOLUTION : 1.2% PARACETAMOL (ALCOHOL FREE)	Strawberry-flavoured clear, pink solution with no apparent crystallisation.	5°C	No change	-	-
		25°C / 60% RH	No change	No change	No change
		40°C / 75% RH	No change	No change	No change.
SOLUTION : 2.4% PARACETAMOL (0.5% ALCOHOL)	Strawberry-flavoured clear, pink solution with no apparent crystallisation.	5°C	No change	-	-
		25°C / 60% RH	No change	No change	No change
		40°C / 75% RH	No change	No change	No change
SOLUTION : 1.2% PARACETAMOL (0.5% ALCOHOL)	Strawberry-flavoured clear, pink solution with no apparent crystallisation.	5°C	No change	-	-
		25°C / 60% RH	No change	No change	No change
		40°C / 75% RH	No change	No change	No change

Table 4 : The appearance of the suspensions over three months of stability testing

APPEARANCE					
PRODUCT	**INITIAL**	**STORAGE TEMPERATURE**	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
SUSPENSION : 2.4% PARACETAMOL	Strawberry-flavoured homogenous, pink suspension with no apparent crystal growth.	5°C	No change	-	-
		25°C / 60% RH	No change	No change	No change
		40°C / 75% RH	Strawberry-flavoured clear, pink solution with large crystals.	Strawberry-flavoured clear, pink solution with large crystals.	Strawberry-flavoured clear, pink solution with large crystals.
MICROFINE SUSPENSION : 2.4% PARACETAMOL	Strawberry-flavoured homogenous, pink suspension with no apparent crystal growth.	5°C	No change	-	-
		25°C / 60% RH	No change	No change	No change
		40°C / 75% RH	Strawberry-flavoured clear, pink solution with large crystals. (Some samples were unaffected.)	Strawberry-flavoured clear, pink solution with large crystals. (Some samples were unaffected.)	Strawberry-flavoured clear, pink solution with large crystals. (Some samples were unaffected.)

Discussion :

No changes in appearance were noted, except for the suspensions that were subjected to 40°C / 75% RH. Amongst these, all of the regular suspension samples were transformed into clear solutions with large crystals. Some, but not all, of the microfine suspensions were affected. It is not clear whether this is due to chance micro-climatic variation within the climatic chambers or whether some other factor (such as pH) renders this product partially immune to the effect.

4.3.2. pH

The pH of each sample was determined at monthly intervals (tables 5 and 6) using a Mettler Toledo pH meter equipped with a glass calomel electrode. Calibration of the pH meter was performed before use with two buffers at pH 4 and pH 7 respectively.

Table 5 : A summary of the pH values of the solutions over three months of stability testing

pH					
PRODUCT	**INITIAL**	**STORAGE TEMPERATURE**	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
SOLUTION : 2.4% PARACETAMOL (ALCOHOL FREE)	4.09	5°C	4.09	-	-
		25°C / 60% RH	4.12	4.14	4.15
		40°C / 75% RH	4.12	4.24	4.20
SOLUTION : 1.2% PARACETAMOL (ALCOHOL FREE)	4.08	5°C	4.16	-	-
		25°C / 60% RH	4.21	4.17	4.19
		40°C / 75% RH	4.15	4.23	4.23
SOLUTION : 2.4% PARACETAMOL (0.5% ALCOHOL)	4.22	5°C	4.20	-	-
		25°C / 60% RH	4.21	4.23	4.22
		40°C / 75% RH	4.38	4.29	4.33
SOLUTION : 1.2% PARACETAMOL (0.5% ALCOHOL)	4.15	5°C	4.16	-	-
		25°C / 60% RH	4.14	4.17	4.15
		40°C / 75% RH	4.17	4.22	4.24

Table 6 : A summary of the pH values of the suspensions over three months of stability testing

pH					
PRODUCT	**INITIAL**	**STORAGE TEMPERATURE**	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
SUSPENSION : 2.4% PARACETAMOL	4.90	5°C	4.97	-	-
		25°C / 60% RH	4.92	4.83	4.83
		40°C / 75% RH	4.71	4.61	4.64
MICROFINE SUSPENSION : 2.4% PARACETAMOL	6.82	5°C	6.87	-	-
		25°C / 60% RH	6.78	6.77	6.80
		40°C / 75% RH	6.72	6.69	6.70

Discussion :

No significant changes in pH were observed.

4.3.3. RELATIVE DENSITY

The relative density of a substance is the ratio of the mass of a given volume of the substance to the mass of an equal volume of water, both weighed at 20°C (BP, 2005:A201-A202).

An Anton Paar DMA38 density meter was used to determine the relative density of each sample at monthly intervals (tables 7 and 8). The device was calibrated daily or before use.

Table 7 : A summary of the relative density of the solutions over three months of stability testing

RELATIVE DENSITY (g/ml)					
PRODUCT	**INITIAL**	**STORAGE TEMPERATURE**	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
SOLUTION : 2.4% PARACETAMOL (ALCOHOL FREE)	1.1563	5°C	1.1562	-	-
		25°C / 60% RH	1.1566	1.1575	1.1578
		40°C / 75% RH	1.1573	1.1579	1.1584
SOLUTION : 1.2% PARACETAMOL (ALCOHOL FREE)	1.1537	5°C	1.1503	-	-
		25°C / 60% RH	1.1600	1.1658	1.1663
		40°C / 75% RH	1.1610	1.1616	1.1612
SOLUTION : 2.4% PARACETAMOL (0.5% ALCOHOL)	1.1735	5°C	1.1533	-	-
		25°C / 60% RH	1.1548	1.1594	1.1594
		40°C / 75% RH	1.1680	1.1586	1.1617
SOLUTION : 1.2% PARACETAMOL (0.5% ALCOHOL)	1.1470	5°C	1.1486	-	-
		25°C / 60% RH	1.1486	1.1500	1.1498
		40°C / 75% RH	1.1482	1.1484	1.1487

Table 8 : A summary of the relative density of the suspensions over three months of stability testing

RELATIVE DENSITY (g/ml)					
PRODUCT	**INITIAL**	**STORAGE TEMPERATURE**	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
SUSPENSION : 2.4% PARACETAMOL	1.1487	5°C	1.0864	-	-
		25°C / 60% RH	1.0851	1.0869	1.0864
		40°C / 75% RH	1.0851	1.0867	1.0867
MICROFINE SUSPENSION : 2.4% PARACETAMOL	1.0819	5°C	1.0816	-	-
		25°C / 60% RH	1.0991	1.1000	1.1005
		40°C / 75% RH	1.0882	1.1005	1.0994

Discussion :

No significant changes in relative density were observed.

4.3.4. PARTICLE SIZE

Particle size distributions in suspension were measured with a Galai-Cis-1 particle size analyser (Israel). This analyser uses dual discipline analysis integrating laser diffraction and image analysis for particle sizing. Samples of the suspension in a suitable dispersing solution (water) were each placed in a small cuvette and the cuvette fitted into the analyser. A small magnetic stirrer inside the cuvette prevented sedimentation of the particles during the measurement. Median diameters by volume are given in table 9.

Table 9 : A summary of the particle size of the suspensions over three months of stability testing

PARTICLE SIZE (µm)*					
PRODUCT	**INITIAL**	**STORAGE TEMPERATURE**	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
SUSPENSION : 2.4% PARACETAMOL	4.94	5°C	4.55	-	-
		25°C / 60% RH	4.85	4.66 (78%)	4.50 (95%)
		40°C / 75% RH	4.18	10.81 (91%)	5.19 (68%)
MICROFINE SUSPENSION : 2.4% PARACETAMOL	5.61 (84%)	5°C	8.77 (82%)	-	-
		25°C / 60% RH	6.30	4.40	4.43 (82%)
		40°C / 75% RH	3.77	3.65	5.06 (85%)

* Confidence level of determination in brackets.

Discussion :

It is worth mentioning that the determination of particle size of some of the paracetamol samples with this specific technique proved to be troublesome, with confidence levels below 95%.

It can be seen that no definite benefit was obtained from formulating a microfine suspension. Though the smallest single reading was obtained for one of these

samples (3.65 µm), most values were on par or even greater than most values obtained for the regular suspension. A likely explanation is that agglomeration occurs to minimise the energy state of individual particles.

4.3.5. VISCOSITY

Table 10 : A summary of the viscosity of the solutions over three months of stability testing

VISCOSITY (cP)					
PRODUCT	**INITIAL**	**STORAGE TEMPERATURE**	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
SOLUTION : 2.4% PARACETAMOL (ALCOHOL FREE)	162.0	5°C	164.0	-	-
		25°C / 60% RH	162.5	163.2	162.4
		40°C / 75% RH	157.0	156.8	158.1
SOLUTION : 1.2% PARACETAMOL (ALCOHOL FREE)	350.4	5°C	292.4	-	-
		25°C / 60% RH	270.4	278.3	278.7
		40°C / 75% RH	314.4	312.2	415.1
SOLUTION : 2.4% PARACETAMOL (0.5% ALCOHOL)	276.9	5°C	295.4	-	-
		25°C / 60% RH	268.9	268.5	267.9
		40°C / 75% RH	313.9	314.6	314.4
SOLUTION : 1.2% PARACETAMOL (0.5% ALCOHOL)	254.9	5°C	259.4	-	-
		25°C / 60% RH	253.4	253.9	254.0
		40°C / 75% RH	245.4	251.3	250.7

Table 11 : A summary of the viscosity of the suspensions over three months of stability testing

VISCOSITY (cP)					
PRODUCT	**INITIAL**	**STORAGE TEMPERATURE**	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
SUSPENSION : 2.4% PARACETAMOL	403.8	5°C	412.2	-	-
		25°C / 60% RH	396.3	396.0	397.2
		40°C / 75% RH	407.8	410.5	408.9
MICROFINE SUSPENSION : 2.4% PARACETAMOL	414.4	5°C	460.4	-	-
		25°C / 60% RH	409.4	408.8	411.3
		40°C / 75% RH	416.9	417.4	417.6

Viscosity was determined with a Brookfield DV-II+ viscometer at a constant temperature of 25°C using a number 2 spindle at 60 r.p.m. Values obtained are given in tables 10 and 11.

Discussion :

No definite relationship was noted between viscosity and the age or storage conditions of the product.

4.3.6. PRESERVATIVE EFFICACY

Samples were sent to the University of Witwatersrand for the determination of preservative efficacy according to the guidelines set by USP 26 (USP 26, 2003: ⟨51⟩2004) for category 3 products. Results are given in tables 12 to 17.

Table 12 : A summary of the preservative efficacy of the alcohol free 2.4% paracetamol solution over three months of stability testing

PRESERVATIVE EFFICACY						
SOLUTION : 2.4% PARACETAMOL (ALCOHOL FREE)						
SAMPLE AGE	**TEST ORGANISM**	**INITIAL INOCULUM**	**LOG UNIT REDUCTION**			**LIMITS FOR USP 26 CATEGORY 3 PRODUCTS**
			DAY7	**DAY14**	**DAY28**	
INITIAL	E. Coli	7.5×10^5	>3.0	>3.0	>3.0	>1.0 log reduction at 14 days & no increase at 28 days
	P. aeruginosa	1.2×10^6	>3.0	>3.0	>3.0	
	S. aureus	2.0×10^5	>3.0	>3.0	>3.0	
	A. niger	1.5×10^5	<1.6	0.5	0.7	No increase from initial inoculum
	C. albicans	2.5×10^5	>3.0	>3.0	>3.0	
3 MONTHS 40°C / 75% RH	E. Coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days
	P. aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
	S. aureus	5.5×10^5	>3.0	>3.0	>3.0	
	A. niger	3.8×10^5	1.4	1.3	2.6	No increase from the initial calculated count at 14 and 28 days
	C. albicans	5.0×10^5	>3.0	>3.0	>3.0	

Table 13 : A summary of the preservative efficacy of the alcohol free 1.2% paracetamol solution over three months of stability testing

PRESERVATIVE EFFICACY						
SOLUTION : 1.2% PARACETAMOL (ALCOHOL FREE)						
SAMPLE AGE	**TEST ORGANISM**	**INITIAL INOCULUM**	**LOG UNIT REDUCTION**			**LIMITS FOR USP 26 CATEGORY 3 PRODUCTS**
			DAY7	**DAY14**	**DAY28**	
INITIAL	E. Coli	7.5×10^5	>3.0	>3.0	>3.0	>1.0 log reduction at 14 days & no increase at 28 days
	P. aeruginosa	1.2×10^6	>3.0	>3.0	>3.0	
	S. aureus	2.0×10^5	>3.0	>3.0	>3.0	
	A. niger	1.5×10^5	<1.6	0.4	0.6	No increase from initial inoculum
	C. albicans	2.5×10^5	>3.0	>3.0	>3.0	
3 MONTHS 40°C / 75% RH	E. Coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days
	P. aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
	S. aureus	5.5×10^5	>3.0	>3.0	>3.0	
	A. niger	3.8×10^5	1.0	0.9	1.1	No increase from the initial calculated count at 14 and 28 days
	C. albicans	5.0×10^5	>3.0	>3.0	>3.0	

Table 14 : A summary of the preservative efficacy of the 2.4% paracetamol solution over three months of stability testing

PRESERVATIVE EFFICACY						
SOLUTION : 2.4% PARACETAMOL (0.5% ALCOHOL)						
SAMPLE AGE	**TEST ORGANISM**	**INITIAL INOCULUM**	**LOG UNIT REDUCTION**			**LIMITS FOR USP 26 CATEGORY 3 PRODUCTS**
			DAY7	**DAY14**	**DAY28**	
INITIAL	E. Coli	7.5×10^5	>3.0	>3.0	>3.0	>1.0 log reduction at 14 days & no increase at 28 days
	P. aeruginosa	1.2×10^6	>3.0	>3.0	>3.0	
	S. aureus	2.0×10^5	>3.0	>3.0	>3.0	
	A. niger	1.5×10^5	<1.6	0.3	0.7	No increase from initial inoculum
	C. albicans	2.5×10^5	>3.0	>3.0	>3.0	
3 MONTHS 40°C / 75% RH	E. Coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days
	P. aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
	S. aureus	5.5×10^5	>3.0	>3.0	>3.0	
	A. niger	3.8×10^5	1.5	1.8	>3.0	No increase from the initial calculated count at 14 and 28 days
	C. albicans	5.0×10^5	>3.0	>3.0	>3.0	

Table 15 : A summary of the preservative efficacy of the 1.2% paracetamol solution over three months of stability testing

PRESERVATIVE EFFICACY						
SOLUTION : 1.2% PARACETAMOL (0.5% ALCOHOL)						
SAMPLE AGE	TEST ORGANISM	INITIAL INOCULUM	LOG UNIT REDUCTION			LIMITS FOR USP 26 CATEGORY 3 PRODUCTS
			DAY7	DAY14	DAY28	
INITIAL	E. Coli	7.5×10^5	>3.0	>3.0	>3.0	>1.0 log reduction at 14 days & no increase at 28 days
	P. aeruginosa	1.2×10^6	>3.0	>3.0	>3.0	
	S. aureus	2.0×10^5	>3.0	>3.0	>3.0	
	A. niger	1.5×10^5	<1.6	0.4	0.5	No increase from initial inoculum
	C. albicans	2.5×10^5	>3.0	>3.0	>3.0	
3 MONTHS 40°C / 75% RH	E. Coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days
	P. aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
	S. aureus	5.5×10^5	>3.0	>3.0	>3.0	
	A. niger	3.8×10^5	1.0	1.3	1.2	No increase from the initial calculated count at 14 and 28 days
	C. albicans	5.0×10^5	>3.0	>3.0	>3.0	

Table 16 : A summary of the preservative efficacy of the 2.4% paracetamol suspension over three months of stability testing

PRESERVATIVE EFFICACY						
SUSPENSION : 2.4% PARACETAMOL						
SAMPLE AGE	**TEST ORGANISM**	**INITIAL INOCULUM**	**LOG UNIT REDUCTION**			**LIMITS FOR USP 26 CATEGORY 3 PRODUCTS**
			DAY7	**DAY14**	**DAY28**	
INITIAL	E. Coli	7.5×10^5	>3.0	>3.0	>3.0	>1.0 log reduction at 14 days & no increase at 28 days
	P. aeruginosa	1.2×10^6	>3.0	>3.0	>3.0	
	S. aureus	2.0×10^5	>3.0	>3.0	>3.0	
	A. niger	1.5×10^5	<1.6	0.3	0.5	No increase from initial inoculum
	C. albicans	2.5×10^5	>3.0	>3.0	>3.0	
3 MONTHS 40°C / 75% RH	E. Coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days
	P. aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
	S. aureus	5.5×10^5	>3.0	>3.0	>3.0	
	A. niger	3.8×10^5	1.0	1.1	0.8	No increase from the initial calculated count at 14 and 28 days
	C. albicans	5.0×10^5	>3.0	>3.0	>3.0	

Table 17 : A summary of the preservative efficacy of the 2.4% paracetamol microfine suspension over three months of stability testing

PRESERVATIVE EFFICACY						
MICROFINE SUSPENSION : 2.4% PARACETAMOL						
SAMPLE AGE	TEST ORGANISM	INITIAL INOCULUM	LOG UNIT REDUCTION			LIMITS FOR USP 26 CATEGORY 3 PRODUCTS
			DAY7	DAY14	DAY28	
INITIAL	E. Coli	7.5×10^5	>3.0	>3.0	>3.0	>1.0 log reduction at 14 days & no increase at 28 days
	P. aeruginosa	1.2×10^6	>3.0	>3.0	>3.0	
	S. aureus	2.0×10^5	>3.0	>3.0	>3.0	
	A. niger	1.5×10^5	<1.6	0.5	0.7	No increase from initial inoculum
	C. albicans	2.5×10^5	>3.0	>3.0	>3.0	
3 MONTHS 40°C / 75% RH	E. Coli	5.0×10^5	>3.0	>3.0	>3.0	Not less than 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days
	P. aeruginosa	1.1×10^6	>3.0	>3.0	>3.0	
	S. aureus	5.5×10^5	>3.0	>3.0	>3.0	
	A. niger	3.8×10^5	<2.6	>3.0	>3.0	No increase from the initial calculated count at 14 and 28 days
	C. albicans	5.0×10^5	<2.7	>3.0	>3.0	

Discussion :

All products complied with USP 26 specifications for category 3 products.

4.3.7. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

4.3.7.1. HPLC analysis of paracetamol concentration

4.3.7.1.1 METHOD

A validated HPLC method for the analysis of paracetamol content of Panado® Paediatric Solution was used. This method was validated on 20.01.98 and was obtained from Adcock Ingram.

The method was adjusted as follows:

- A Luna C_{18} (250.0 x 4.6 mm) was used instead of a Waters Novapak C_{18} (150.0 x 3.9). This was done to improve peak resolution in the samples.
- Sample preparation : Double the amount of sample was weighed to compensate for an additional step during which the sample was mixed with methanol and centrifuged to eliminate xanthan gum from the solution. This was done because xanthan gum would accumulate in the column and cause increased system pressure and eventual blockage.

Instrument :

HPLC system equipped with a variable wavelength UV detector and an integrator, or equivalent. In this instance a Hewlett Packard 1100 equipped with a variable wavelength UV detector and an integrator was used.

Instrument parameters :

Column : Phenomenex Luna C_{18} (5µm) (250.0 x 4.6 mm)

Mobile phase : 30 : 70 Acetonitrile : water. Filter through a 0.45 µm filter.

Flow rate : 1.0 ml / minute

Injection volume : 5 µl

Wavelength : 280 nm

Retention time : Paracetamol – About 4.1 minutes.

Standard preparation :

For full-strength standards : Accurately weigh 0.240 g of paracetamol standard and transfer quantitatively into a 50 ml volumetric flask. Dissolve in and dilute to volume with methanol. Pipette 25 ml into a 100 ml volumetric flask and dilute to volume with mobile phase. Filter through a 0.45 µm membrane filter.

For half-strength standards : Accurately weigh 0.120 g of paracetamol standard and transfer quantitatively into a 50 ml volumetric flask. Dissolve in and dilute to volume with methanol. Pipette 25 ml into a 100 ml volumetric flask and dilute to volume with mobile phase. Filter through a 0.45 µm membrane filter.

Sample preparation :

Accurately weigh 10 ml of the sample into a 50 ml volumetric flask. Dissolve in and dilute to volume with methanol. Decant the liquid into centrifuge tubes and centrifuge at 5000 r.p.m. for 30 minutes. Pipette 25 ml of the clear liquid into a 100 ml volumetric flask and dilute to volume with mobile phase.

Calculation :

$$\frac{A_U \times WEIGHT\ STANDARD\,(mg) \times R.D. \times 5 \times \%PURITY}{A_S \times WEIGHT\ SAMPLE\,(g) \times 100} = mg\ PARACETAMOL / 5ml$$

Where :

- A_U = Area of sample peak
- A_S = Area of standard peak
- **R.D.** = Relative density of sample
- **%Purity** = Purity of the standard (%)

4.3.7.1.2 RESULTS

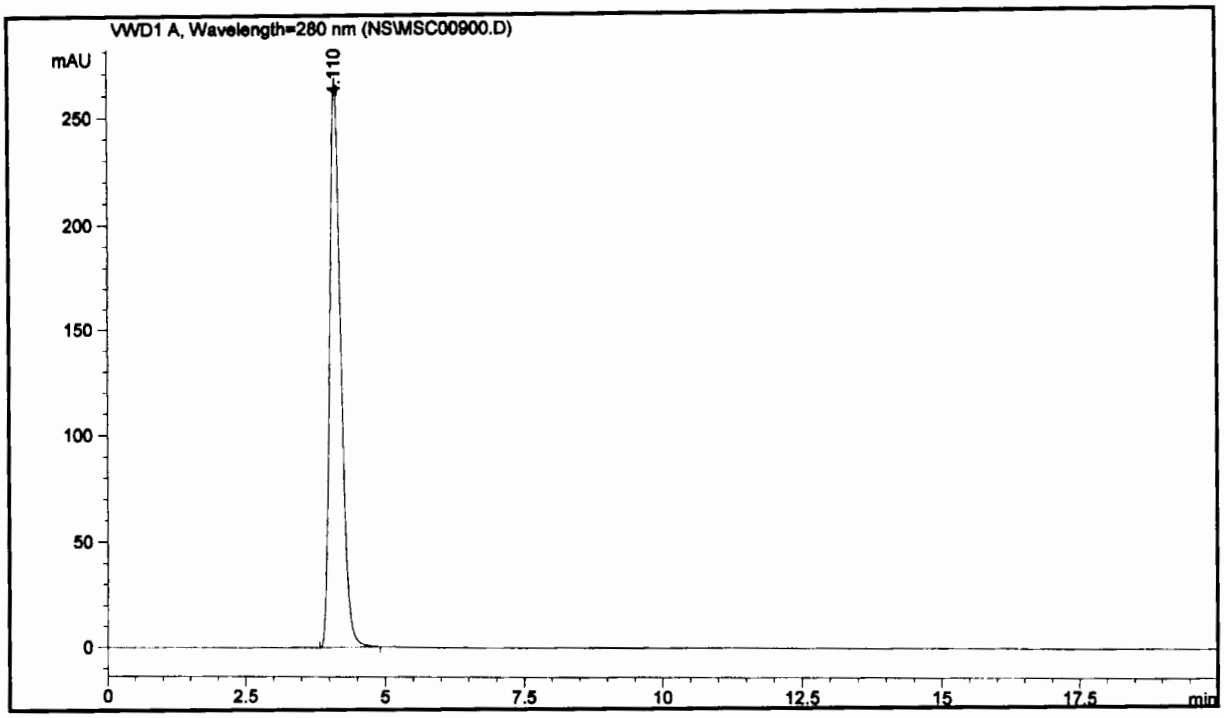


Figure 1 : A chromatogram of the paracetamol standard

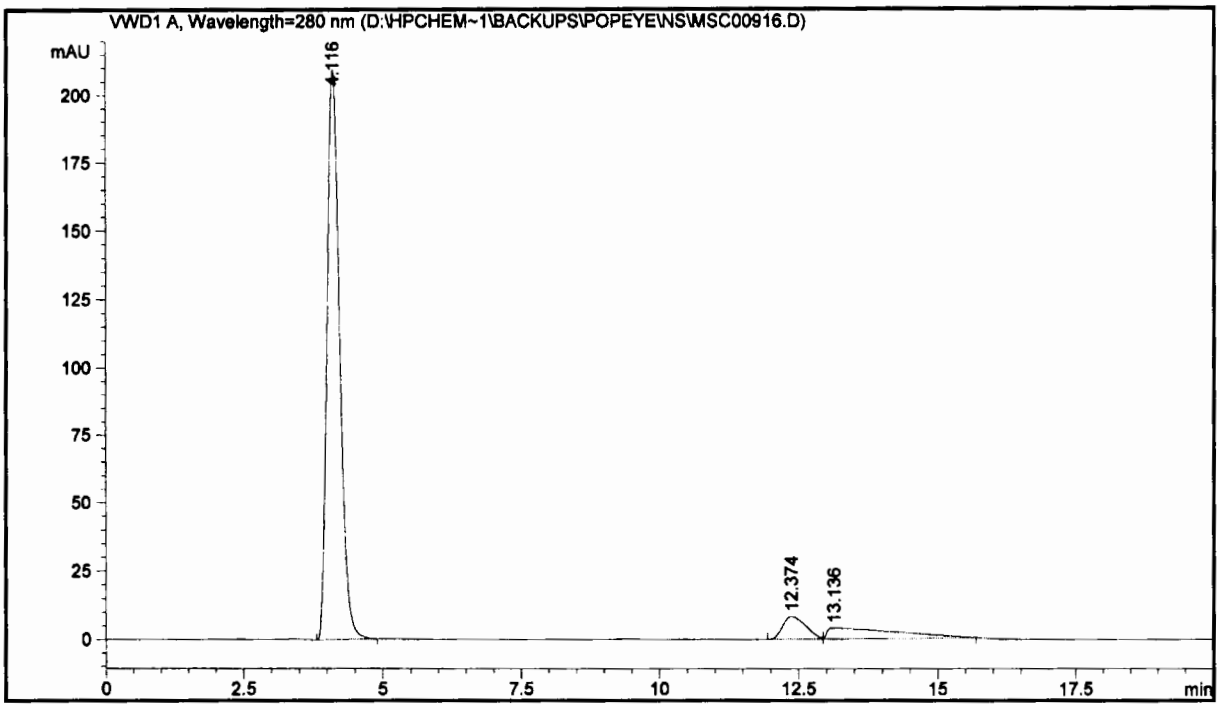


Figure 2 : A chromatogram of a sample

A summary of paracetamol concentration for the six formulations over three months of stability testing is given in tables 18 and 19. No direct correlation between age or storage conditions and decreasing concentration was shown.

Storage conditions did have a major influence on the two suspensions. The paracetamol in some samples subjected to 40°C / 75% RH dissolved completely and these products were clear solutions when removed from the climatic chamber. Recrystallisation occurred within 24 hours. The new crystal growth resulted in a clear product with very large crystals of approximately 2 – 4 mm in diameter (figures 3 and 4). Infra-red and DSC tests confirmed that these were paracetamol crystals. The crystals were not included during sample preparation for HPLC. Therefore, the values obtained only indicate the amount of paracetamol remaining in solution. It is assumed that not all samples were affected in this way, because of micro-climatic variation within the climatic chamber.

Figure 3 : A suspension subjected to 40°C / 75% RH

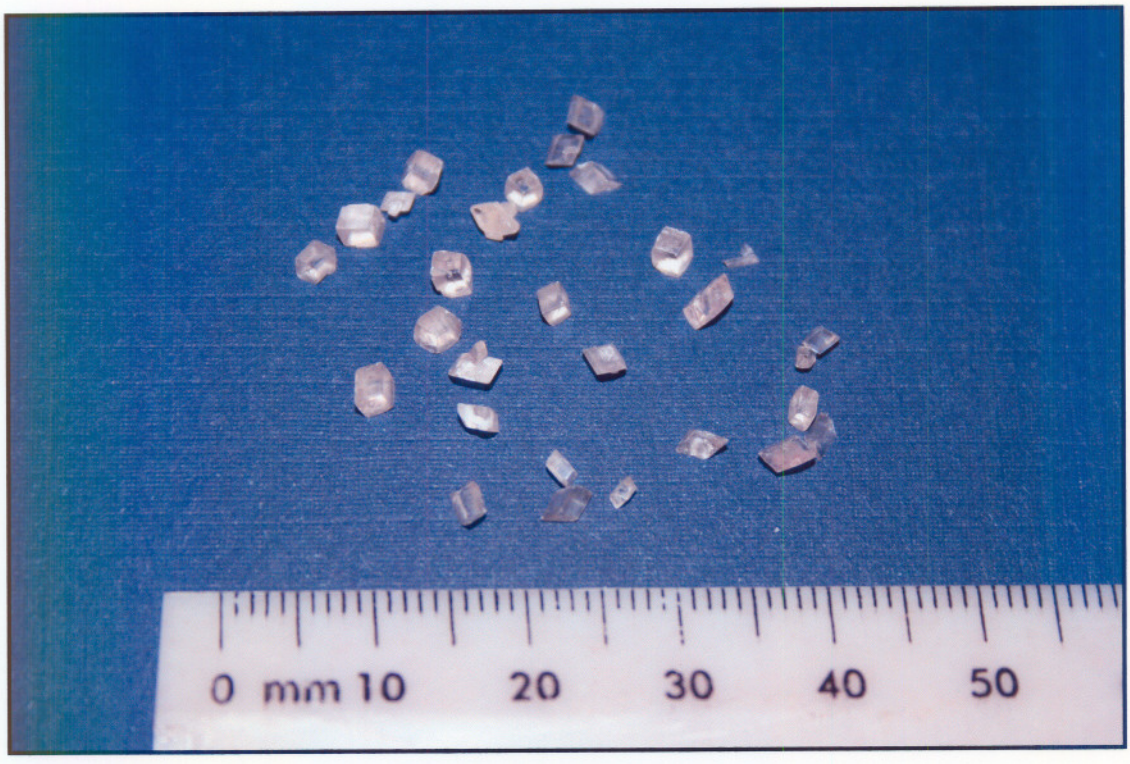


Figure 4 : Paracetamol crystals

Table 18 : Paracetamol content of the solutions over three months of stability testing

HPLC : PARACETAMOL					
PRODUCT	**INITIAL**	**STORAGE TEMPERATURE**	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
SOLUTION : 2.4% PARACETAMOL (ALCOHOL FREE)	101.78%	5°C	105.33%	-	-
		25°C / 60% RH	107.78%	101.03%	102.48%
		40°C / 75% RH	103.67%	101.68%	102.04%
SOLUTION : 1.2% PARACETAMOL (ALCOHOL FREE)	98.92%	5°C	104.38%	-	-
		25°C / 60% RH	112.32%	108.28%	100.13%
		40°C / 75% RH	108.87%	101.58%	109.25%
SOLUTION : 2.4% PARACETAMOL (0.5% ALCOHOL)	102.24%	5°C	99.73%	-	-
		25°C / 60% RH	108.46%	100.55%	99.19%
		40°C / 75% RH	110.60%	101.91%	100.00%
SOLUTION : 1.2% PARACETAMOL (0.5% ALCOHOL)	104.82%	5°C	109.75%	-	-
		25°C / 60% RH	111.63%	101.38%	102.07%
		40°C / 75% RH	105.85%	103.33%	101.82%

Table 19 : Paracetamol content of the suspensions over three months of stability testing

HPLC : PARACETAMOL					
PRODUCT	**INITIAL**	**STORAGE TEMPERATURE**	**1 MONTH**	**2 MONTHS**	**3 MONTHS**
SUSPENSION : 2.4% PARACETAMOL	102.55%	5°C	111.05%	-	-
		25°C / 60% RH	105.93%	103.25%	103.78%
		40°C / 75% RH	66.05%	68.68%	74.89%
MICROFINE SUSPENSION : 2.4% PARACETAMOL	101.86%	5°C	100.15%	-	-
		25°C / 60% RH	112.30%	109.31%	103.83%
		40°C / 75% RH	65.41%	101.93%	102.47%

Values for most samples did not fall within 2% of predicted values and variation was substantial. This is probably due to shortcomings of the method rather than an accurate representation of actual paracetamol content of the samples.

During sample preparation the following problem was encountered : When methanol was added to the sample, gas bubbles were trapped in the resulting xanthan gum mass. Variation in the amount of gas trapped, resulted in variation of volume, and therefore concentration, of the samples. Unfortunately this step is crucial to the execution of the process. If not removed from the samples, xanthan gum was found to accumulate in the column. The result was increased pressure and eventual system failure. Also, the damage to columns was irreversible, resulting in exorbitant costs. The use of pre-columns was investigated, but these were unpractical because they would block completely after just a few injections. It also proved impossible to filter samples using 0.45 µm filters – after allowing two drops of sample through, blockage would cause the filters to shoot off the end of the syringe.

More detailed HPLC results for individual months and products are given in tables 20 - 38. It is shown that variation between duplicate samples of the same product is very large in some cases (up to 10.21%). This is a further indication that this method cannot be consistently executed in the altered form in which it was used for this study.

Table 20: Initial paracetamol content of the six products

HPLC : PARACETAMOL (INITIAL)						
PRODUCT	**DUPLICATE SAMPLES (mg/5ml)**		**DUPLICATE SAMPLES (%)**		**VARIATION (%)**	**AVERAGE (%)**
SOLUTION : 2.4% PARACETAMOL (ALCOHOL FREE)	121.27	123.00	101.06	102.50	1.43	101.78
SOLUTION : 1.2% PARACETAMOL (ALCOHOL FREE)	58.20	60.50	97.00	100.83	3.95	98.92
SOLUTION : 2.4% PARACETAMOL (0.5% ALCOHOL)	122.98	122.40	102.48	102.00	0.47	102.24
SOLUTION : 1.2% PARACETAMOL (0.5% ALCOHOL)	64.44	61.34	107.40	102.23	5.05	104.82
SUSPENSION : 2.4% PARACETAMOL	120.76	125.36	100.63	104.47	3.81	102.55
MICROFINE SUSPENSION : 2.4% PARACETAMOL	123.62	120.83	103.02	100.69	2.31	101.86

Table 21: Paracetamol content of the alcohol free 2.4% paracetamol solution after the first month of stability testing

HPLC : PARACETAMOL (1 MONTH)						
SOLUTION : 2.4% PARACETAMOL (ALCOHOL FREE)						
STORAGE TEMPERATURE	DUPLICATE SAMPLES (mg/5ml)		DUPLICATE SAMPLES (%)		VARIATION (%)	AVERAGE (%)
5°C	127.80	124.99	106.50	104.16	2.25	105.33
25°C / 60% RH	128.70	129.98	107.25	108.32	1.00	107.78
40°C / 75% RH	123.62	125.18	103.02	104.32	1.26	103.67

Table 22: Paracetamol content of the alcohol free 1.2% paracetamol solution after the first month of stability testing

HPLC : PARACETAMOL (1 MONTH)						
SOLUTION : 1.2% PARACETAMOL (ALCOHOL FREE)						
STORAGE TEMPERATURE	DUPLICATE SAMPLES (mg/5ml)		DUPLICATE SAMPLES (%)		VARIATION (%)	AVERAGE (%)
5°C	61.39	63.86	102.32	106.43	4.02	104.38
25°C / 60% RH	65.72	69.06	109.53	115.10	5.08	112.32
40°C / 75% RH	65.59	65.05	109.32	108.42	0.83	108.87

Table 23: Paracetamol content of the 2.4% paracetamol solution after the first month of stability testing

HPLC : PARACETAMOL (1 MONTH)						
SOLUTION : 2.4% PARACETAMOL (0.5% ALCOHOL)						
STORAGE TEMPERATURE	**DUPLICATE SAMPLES (mg/5ml)**		**DUPLICATE SAMPLES (%)**		**VARIATION (%)**	**AVERAGE (%)**
5°C	125.49	113.87	104.58	94.89	10.21	99.73
25°C / 60% RH	129.83	130.47	108.19	108.73	0.49	108.46
40°C / 75% RH	132.04	133.39	110.03	111.16	1.02	110.60

Table 24: Paracetamol content of the 1.2% paracetamol solution after the first month of stability testing

HPLC : PARACETAMOL (1 MONTH)						
SOLUTION : 1.2% PARACETAMOL (0.5% ALCOHOL)						
STORAGE TEMPERATURE	**DUPLICATE SAMPLES (mg/5ml)**		**DUPLICATE SAMPLES (%)**		**VARIATION (%)**	**AVERAGE (%)**
5°C	66.82	64.87	111.37	108.12	3.01	109.75
25°C / 60% RH	66.58	67.37	110.97	112.28	1.19	111.63
40°C / 75% RH	63.95	63.07	106.58	105.12	1.40	105.85

Table 25: Paracetamol content of the 2.4% paracetamol suspension after the first month of stability testing

HPLC : PARACETAMOL (1 MONTH)						
SUSPENSION : 2.4% PARACETAMOL						
STORAGE TEMPERATURE	**DUPLICATE SAMPLES (mg/5ml)**		**DUPLICATE SAMPLES (%)**		**VARIATION (%)**	**AVERAGE (%)**
5°C	132.31	134.21	110.26	111.84	1.44	111.05
25°C / 60% RH	128.00	126.24	106.67	105.20	1.39	105.93
40°C / 75% RH	78.43	80.09	65.36	66.74	2.12	66.05

Table 26: Paracetamol content of the micro fine 2.4% paracetamol suspension after the first month of stability testing

HPLC : PARACETAMOL (1 MONTH)						
MICROFINE SUSPENSION : 2.4% PARACETAMOL						
STORAGE TEMPERATURE	**DUPLICATE SAMPLES (mg/5ml)**		**DUPLICATE SAMPLES (%)**		**VARIATION (%)**	**AVERAGE (%)**
5°C	121.19	119.17	100.99	99.31	1.70	100.15
25°C / 60% RH	133.83	135.69	111.53	113.08	1.39	112.30
40°C / 75% RH	78.78	78.19	65.65	65.16	0.76	65.41

Table 27: Paracetamol content of the alcohol free 2.4% paracetamol solution after the second month of stability testing

HPLC : PARACETAMOL (2 MONTHS)						
SOLUTION : 2.4% PARACETAMOL (ALCOHOL FREE)						
STORAGE TEMPERATURE	DUPLICATE SAMPLES (mg/5ml)		DUPLICATE SAMPLES (%)		VARIATION (%)	AVERAGE (%)
5°C	-	-	-	-	-	-
25°C / 60% RH	121.10	121.37	100.92	101.14	0.22	101.03
40°C / 75% RH	122.04	121.98	101.70	101.65	0.05	101.68

Table 28: Paracetamol content of the alcohol free 1.2% paracetamol solution after the second month of stability testing

HPLC : PARACETAMOL (2 MONTHS)						
SOLUTION : 1.2% PARACETAMOL (ALCOHOL FREE)						
STORAGE TEMPERATURE	DUPLICATE SAMPLES (mg/5ml)		DUPLICATE SAMPLES (%)		VARIATION (%)	AVERAGE (%)
5°C	-	-	-	-	-	-
25°C / 60% RH	65.14	64.80	108.57	108.00	0.53	108.28
40°C / 75% RH	61.42	60.47	102.37	100.78	1.57	101.58

Table 29: Paracetamol content of the 2.4% paracetamol solution after the second month of stability testing

HPLC : PARACETAMOL (2 MONTHS)						
SOLUTION : 2.4% PARACETAMOL (0.5% ALCOHOL)						
STORAGE TEMPERATURE	**DUPLICATE SAMPLES (mg/5ml)**		**DUPLICATE SAMPLES (%)**		**VARIATION (%)**	**AVERAGE (%)**
5°C	-	-	-	-	-	-
25°C / 60% RH	120.49	120.82	100.41	100.68	0.27	100.55
40°C / 75% RH	121.50	123.07	101.25	102.56	1.29	101.91

Table 30: Paracetamol content of the 1.2% paracetamol solution after the second month of stability testing

HPLC : PARACETAMOL (2 MONTHS)						
SOLUTION : 1.2% PARACETAMOL (0.5% ALCOHOL)						
STORAGE TEMPERATURE	**DUPLICATE SAMPLES (mg/5ml)**		**DUPLICATE SAMPLES (%)**		**VARIATION (%)**	**AVERAGE (%)**
5°C	-	-	-	-	-	-
25°C / 60% RH	60.86	60.79	101.43	101.32	0.12	101.38
40°C / 75% RH	61.50	62.49	102.50	104.15	1.61	103.33

Table 31: Paracetamol content of the 2.4% paracetamol suspension after the second month of stability testing

HPLC : PARACETAMOL (2 MONTHS)						
SUSPENSION : 2.4% PARACETAMOL						
STORAGE TEMPERATURE	**DUPLICATE SAMPLES (mg/5ml)**		**DUPLICATE SAMPLES (%)**		**VARIATION (%)**	**AVERAGE (%)**
5°C	-	-	-	-	-	-
25°C / 60% RH	123.75	124.05	103.13	103.38	1.39	105.93
40°C / 75% RH	79.21	85.62	66.01	71.35	8.09	68.68

Table 32: Paracetamol content of the micro fine 2.4% paracetamol suspension after the second month of stability testing

HPLC : PARACETAMOL (2 MONTHS)						
MICROFINE SUSPENSION : 2.4% PARACETAMOL						
STORAGE TEMPERATURE	**DUPLICATE SAMPLES (mg/5ml)**		**DUPLICATE SAMPLES (%)**		**VARIATION (%)**	**AVERAGE (%)**
5°C	-	-	-	-	-	-
25°C / 60% RH	130.99	131.34	109.16	109.45	0.27	109.31
40°C / 75% RH	120.75	123.86	100.63	103.22	2.58	101.93

Table 33: Paracetamol content of the alcohol free 2.4% paracetamol solution after the third month of stability testing

HPLC : PARACETAMOL (3 MONTHS)						
SOLUTION : 2.4% PARACETAMOL (ALCOHOL FREE)						
STORAGE TEMPERATURE	**DUPLICATE SAMPLES (mg/5ml)**		**DUPLICATE SAMPLES (%)**		**VARIATION (%)**	**AVERAGE (%)**
5°C	-	-	-	-	-	-
25°C / 60% RH	123.89	122.06	103.24	101.72	1.50	102.48
40°C / 75% RH	122.58	122.32	102.15	101.93	0.21	102.04

Table 34: Paracetamol content of the alcohol free 1.2% paracetamol solution after the third month of stability testing

HPLC : PARACETAMOL (3 MONTHS)						
SOLUTION : 1.2% PARACETAMOL (ALCOHOL FREE)						
STORAGE TEMPERATURE	**DUPLICATE SAMPLES (mg/5ml)**		**DUPLICATE SAMPLES (%)**		**VARIATION (%)**	**AVERAGE (%)**
5°C	-	-	-	-	-	-
25°C / 60% RH	60.24	59.92	100.40	99.87	0.53	100.13
40°C / 75% RH	65.27	65.82	108.78	109.70	0.84	109.25

Table 35: Paracetamol content of the 2.4% paracetamol solution after the third month of stability testing

HPLC : PARACETAMOL (3 MONTHS)						
SOLUTION : 2.4% PARACETAMOL (0.5% ALCOHOL)						
STORAGE TEMPERATURE	DUPLICATE SAMPLES (mg/5ml)		DUPLICATE SAMPLES (%)		VARIATION (%)	AVERAGE (%)
5°C	-	-	-	-	-	-
25°C / 60% RH	119.18	118.87	99.32	99.06	0.26	99.19
40°C / 75% RH	119.78	120.22	99.82	100.18	0.37	100.00

Table 36: Paracetamol content of the 1.2% paracetamol solution after the third month of stability testing

HPLC : PARACETAMOL (3 MONTHS)						
SOLUTION : 1.2% PARACETAMOL (0.5% ALCOHOL)						
STORAGE TEMPERATURE	DUPLICATE SAMPLES (mg/5ml)		DUPLICATE SAMPLES (%)		VARIATION (%)	AVERAGE (%)
5°C	-	-	-	-	-	-
25°C / 60% RH	61.46	61.02	102.43	101.70	0.72	102.07
40°C / 75% RH	61.15	61.03	101.92	101.72	0.20	101.82

Table 37: Paracetamol content of the 2.4% paracetamol suspension after the third month of stability testing

HPLC : PARACETAMOL (3 MONTHS)						
SUSPENSION : 2.4% PARACETAMOL						
STORAGE TEMPERATURE	DUPLICATE SAMPLES (mg/5ml)		DUPLICATE SAMPLES (%)		VARIATION (%)	AVERAGE (%)
5°C	-	-	-	-	-	-
25°C / 60% RH	124.44	124.61	103.70	103.84	0.14	103.78
40°C / 75% RH	86.72	93.02	72.27	77.52	7.27	74.89

Table 38: Paracetamol content of the micro fine 2.4% paracetamol suspension after the third month of stability testing

HPLC : PARACETAMOL (3 MONTHS)						
MICROFINE SUSPENSION : 2.4% PARACETAMOL						
STORAGE TEMPERATURE	DUPLICATE SAMPLES (mg/5ml)		DUPLICATE SAMPLES (%)		VARIATION (%)	AVERAGE (%)
5°C	-	-	-	-	-	-
25°C / 60% RH	124.59	124.61	103.83	103.84	0.02	103.83
40°C / 75% RH	124.05	121.87	103.38	101.56	1.79	102.47

4.3.7.1.3 CONCLUSION

An altered version of a validated method was used to determine the paracetamol content of the six products. The changes to the method was essential, but gave rise to an unavoidable complication during sample preparation. The results obtained weren't reproducible and variation between duplicate samples was substantial in many instances. Therefore, the results can only give an indication of product stability and paracetamol content, but the values obtained cannot be taken as absolute.

It was found that the paracetamol in suspensions kept at 40°C / 75% RH dissolved completely to yield clear solutions. This is due to the higher solubility of paracetamol at higher temperatures. When these solutions cooled to room temperature, they became over-saturated and recrystallisation of the paracetamol occurred. Thus, paracetamol suspensions should be protected from excessive heat.

4.3.7.2. HPLC analysis of 4-aminophenol concentration

4.3.7.2.1 METHOD

A validated HPLC method for the analysis of 4-aminophenol content of Panado® Paediatric Solution was used. This method was validated on 20.01.98 and was obtained from Adcock Ingram.

The method was adjusted as follows:

- Sample preparation : Double the amount of sample was weighed to compensate for an additional step during which the sample was mixed with methanol and centrifuged to eliminate xanthan gum from the solution. This was done because xanthan gum would accumulate in the column and cause increased system pressure and eventual blockage.

Instrument :

HPLC system equipped with a variable wavelength UV detector and an integrator, or equivalent. In this instance a Hewlett Packard 1100 equipped with a variable wavelength UV detector and an integrator was used.

Instrument parameters :

Column : Phenomenex Luna C_{18} (5µm) (250.0 x 4.6 mm)

Mobile phase : 10 : 90 : 0.4 methanol : 0.01M B5* : formic acid. Filter through a 0.45 µm filter.

Flow rate : 1.0 ml / minute

Injection volume : 20 µl

Wavelength : 272 nm

Retention time : 4-aminophenol – About 4.7 minutes.

* B5 is Na-pentane sulphonic salt

Standard preparation :

For full-strength standards : Accurately weigh 60 mg of 4-aminophenol standard and transfer quantitatively into a 100 ml volumetric flask. Dissolve in and dilute to volume with mobile phase. Pipette 1 ml into a second 100 ml volumetric flask and dilute to volume with mobile phase. Filter through a 0.45 µm membrane filter.

For half-strength standards : Accurately weigh 30 mg of 4-aminophenol standard and transfer quantitatively into a 100 ml volumetric flask. Dissolve in and dilute to volume with mobile phase. Pipette 1 ml into a second 100 ml volumetric flask and dilute to volume with mobile phase. Filter through a 0.45 µm membrane filter.

Sample preparation :

Accurately weigh 10 ml of the sample into a 50 ml volumetric flask. Dissolve in and dilute to volume with methanol. Decant the liquid into centrifuge tubes and centrifuge at 5000 rpm for 30 minutes. Pipette 25 ml of the clear liquid into a 100 ml volumetric flask and dilute to volume with mobile phase.

Calculation :

$$\frac{A_U \times WEIGHT\,STANDARD\,(mg) \times R.D. \times 5}{A_S \times WEIGHT\,SAMPLE\,(g) \times A} = \%\ 4-AMINOPHENOL\,OF\,PARACETAMOL\,CONTENT$$

Where :

- **A_U** = Area of sample peak
- **A_S** = Area of standard peak
- **R.D.** = Relative density of sample
- **A** = Content of paracetamol in assay

4.3.7.2.2 RESULTS

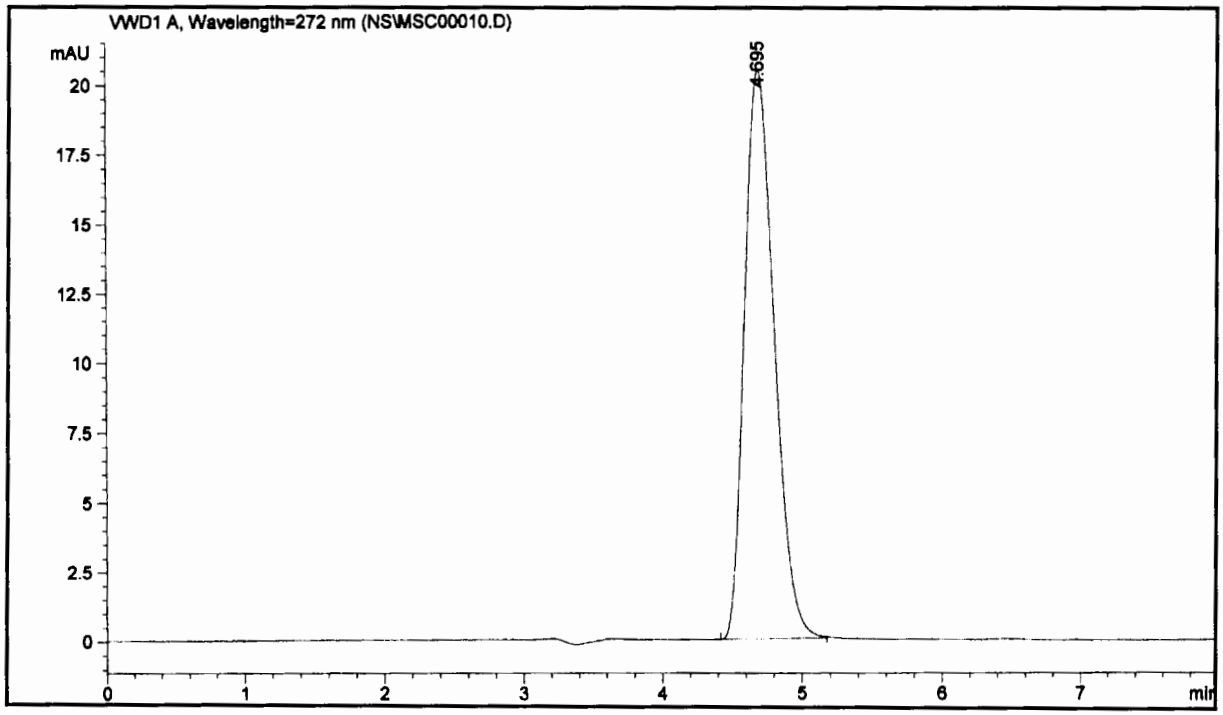


Figure 5 : A chromatogram of the 4-aminophenol standard

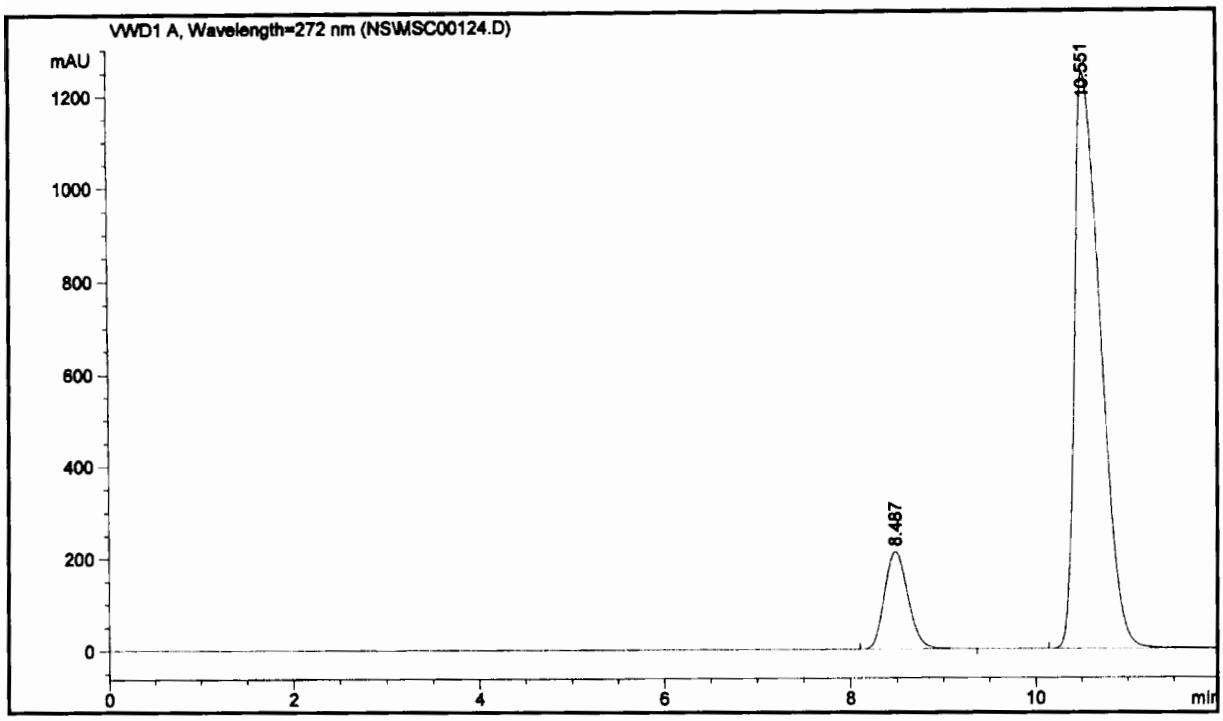


Figure 6 : A chromatogram of a sample

No 4-aminophenol peaks were detected in any of the samples.

4.3.7.2.3 CONCLUSION

No 4-aminophenol peaks were detected. Thus, degradation of paracetamol to 4-aminophenol could not be shown.

4.3.7.3. HPLC analysis of preservative concentration

4.3.7.3.1 METHOD

A validated HPLC method for the analysis of methyl hydroxybenzoate and propyl hydoxybenzoate content of Anti Pain® Solution was used. This method was obtained from Aspen Pharmacare Limited.

The method was adjusted as follows:

- Sample preparation : Double the amount of sample was weighed to compensate for an additional dilution step. No water was added and the sample was centrifuged to eliminate xanthan gum from the solution. This was done because xanthan gum would accumulate in the column and cause increased system pressure and eventual blockage. Also, the sample could not be filtered through a 0.45 µm filter.

Instrument :

HPLC system equipped with a variable wavelength UV detector and an integrator, or equivalent. In this instance a Hewlett Packard 1100 equipped with a variable wavelength UV detector and an integrator was used.

Instrument parameters :

Column	: Symmetry C_{18} (5µm) (150 x 3.9 mm) or equivalent.
Mobile phase	: 35 : 65 acetonitrile : water with 1% acetic acid. Filter through a 0.45 µm filter.
Flow rate	: 1.0 ml / minute
Injection volume	: 10 µl
Wavelength	: 254 nm
Retention time	: Methyl hydroxybenzoate – About 3.5 minutes.
	: Propyl hydoxybenzoate - About 10.0 minutes.

Standard preparation :

Accurately weigh 100 mg of methyl hydroxybenzoate and 10 mg of propyl hydroxybenzoate standard and transfer quantitatively into a single 100 ml volumetric flask. Add 5 ml methanol and sonicate for 2 minutes. Make up to volume with methanol and mix well. Dilute 5 ml to 50 ml with methanol.

Sample preparation :

Transfer 10 ml of sample to a 50 ml volumetric flask. Dissolve in and dilute to volume with methanol. Decant the liquid into centrifuge tubes and centrifuge at 5000 rpm for 30 minutes. Pipette 25 ml of the clear liquid into a 50 ml volumetric flask and dilute to volume with methanol. Filter the solution through a 0.45 µm filter, discarding the first 10 ml of filtrate.

Calculations :

$$\%m/v\,METHYL\,HYDROXYBENZOATE = \frac{P2 \times MASS\,STD1 \times C1}{P1 \times 100\,000}$$

$$\%m/v\,PROPYL\,HYDROXYBENZOATE = \frac{P4 \times MASS\,STD2 \times C2}{P3 \times 100\,000}$$

Where :

- **P1** = Area of the methyl hydroxybenzoate standard peak
- **P2** = Area of the methyl hydroxybenzoate sample peak
- **P3** = Area of the propyl hydroxybenzoate standard peak
- **P4** = Area of the propyl hydroxybenzoate sample peak
- **MASS STD 1** = Mass of methyl hydroxybenzoate standard used, expressed in mg.
- **MASS STD 2** = Mass of propyl hydroxybenzoate standard used, expressed in mg.
- **C1** = Potency of the methyl hydroxybenzoate standard, expressed as a percentage.
- **C2** = Potency of the propyl hydroxybenzoate standard, expressed as a percentage.

4.3.7.3.2 RESULTS

Due to repeated attempts to determine the paracetamol and 4-aminophenol content of the products, all samples available for HPLC analysis were exhausted before preservative content could be determined.

It can be safely assumed that should these assays have been performed, the same difficulties experienced in the previous two methods, would also have caused problems here. Any results obtained would have had doubtful value.

Preservative efficacy was determined and all samples complied with USP 26 specifications for category 3 products. In this case, these results are a better indication of the preservative content and efficacy.

CHAPTER 5

CONCLUSION

Paracetamol is currently the analgesic / antipyretic which is most commonly used. It is inexpensive, available without prescription and is relatively safe in therapeutic doses. Since toxic effects are so rare, paracetamol is the drug of choice for treating pain and fever in children. Its analgesic and antipyretic effects are comparable to that of aspirin, but paracetamol is safe in many instances where the use of aspirin may be contra-indicated: aspirin allergy; haemophilia; history of peptic ulcers; where bronchospasm is precipitated by aspirin, and viral conditions in children.

The poor water-solubility of paracetamol, as well as its bitter taste, complicates the formulation of oral paediatric preparations.

The physico-chemical properties of paracetamol, as well as its formulation into liquid dosage forms were studied during preformulation. It has been shown that ethanol increases the risk of paracetamol-induced hepatotoxicity and that its use should be avoided. Various co-solvents were thus considered as alternative to ethanol for use in aiding the solubility of paracetamol.

The effect of meglumine on paracetamol solubility was briefly investigated and it did indeed improve water solubility markedly. However, the pH values of these solutions were so high that meglumine is rendered totally useless as a co-solvent for paracetamol. Further investigations were abandoned.

General methods for the formulation of liquid paracetamol dosage forms were developed during early formulation.

In total, twenty-four oral paediatric paracetamol preparations were formulated. Six of these were selected to undergo stability testing. Selection was based on appearance, potential stability, as observed over a few days' storage at room temperature, and patient acceptability. Those formulations that were selected for stability trials included:

- Suspensions
    - Formula 1.

- Micro-fine suspensions
    - Formula 1.
- Solutions
    - Formula 18: Containing 2.40 g / 100 ml paracetamol and no ethanol;
    - Formula 19: Containing 1.20 g / 100 ml paracetamol and no ethanol;
    - Formula 20: Containing 2.40 g / 100 ml paracetamol and 0.50% v/v ethanol; and
    - Formula 21: Containing 1.20 g / 100 ml paracetamol and 0.50% v/v ethanol.

These four solutions all contained the same co-solvent systems and differed only in their paracetamol and ethanol contents. This was done in order to determine, by comparison during stability testing, the possible effects that 0.50% v/v of ethanol and varying paracetamol concentrations would have on the stability of these solutions.

The selected samples were subjected to the following storage conditions before being subjected to monthly stability tests:

- 5°C ± 3°C
- 25°C ± 2°C / 60% RH ± 5% RH
- 40°C ± 2°C / 75% RH ± 5% RH

No significant changes in pH, relative density or viscosity were observed. Preservative efficacy was determined according to USP 26 specifications for category 3 products and all samples complied.

Visual assessment of the samples revealed that only the suspensions stored at 40°C ± 2°C / 75% RH ± 5% RH underwent a change in appearance. Because of paracetamol's increased solubility at higher temperatures, it is essential that suspensions be kept at room temperature (25°C). Paracetamol suspensions are much cheaper to produce than solutions and this makes them an attractive option for State use in developing countries. However, many of these poorer nations are in Africa and the East where the actual maximum ambient temperature may be in excess of 40°C – suspended paracetamol particles will dissolve during the day and recrystallise into large crystals at night.

Determining particle size of some of the suspension samples proved troublesome, with confidence levels below 95%. It was seen that no definite benefit was gained from formulating a microfine suspension. Though the smallest single reading was obtained for one of these samples (3.65), most values were on par or even greater than most values obtained for the regular suspension.

Altered versions of validated HPLC methods were used to determine the paracetamol and 4-aminophenol content of the six products. The changes to the methods were essential to remove xanthan gum from the test samples, but gave rise to an unavoidable complication during sample preparation. If not removed from the samples, xanthan gum was found to accumulate in the column. Much effort was spent in an attempt to correct the problem, but the simple solution is probably to exclude xanthan gum from all products that will undergo HPLC testing.

The results obtained from the paracetamol assay weren't reproducible and variation between duplicate samples was substantial in many instances. Therefore, the results can only give an indication of product stability and paracetamol content, but the values obtained cannot be taken as absolute. No 4-aminophenol peaks were detected. Hence, degradation of paracetamol to 4-aminophenol could not be shown.

Though no definite conclusion as to the stability regarding paracetamol content can be made without accurate HPLC results, it appears as though all of the products formulated were stable at room temperature. Furthermore, it could not be shown that either halving the conventional paracetamol concentration (2.4 g / 100 ml), or adding 0.5% v/v ethanol to a formulation had any beneficial effects on product stability. Thus, formulating alcohol-free 2.4 g / 100 ml paracetamol solutions and suspensions can be achieved by using alternative co-solvents in amounts not exceeding their recommended concentrations.

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