

DEVELOPMENT AND EVALUATION OF A COMBINATION ANALGESIC CAPSULE

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“The heights by great men reached and kept, were not obtained by sudden flight. But they, while their companions slept, were toiling upward in the night.”

Henry Wadsworth

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CHAPTER 1

PAIN MANAGEMENT - AIMS AND OBJECTIVES

INTRODUCTION

Pain is an unpleasant sensation that originates from ongoing or impeding tissue damage. The word “pain” comes from the Latin word *peona* meaning punishment or penalty (Guidon *et al.*, 2007:2121).

Humans have always known and sought relief from pain. The International Association for the Study of Pain (1979:249-252) has defined pain as:

An unpleasant sensory or emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is always subjective. Each individual learns the application of the word through experience related to injury in early life. It is unquestionably a sensation in a part of the body, but it is also unpleasant, and therefore also an emotional experience. Many people report pain in the absence of tissue damage or any likely pathophysiological cause; usually this happens for psychological reasons. There is no way to distinguish their experience from that due to tissue damage.

Pain can be acute or chronic in nature. Physiologically, pain may be divided into nociceptive pain, and neuropathic pain. Often nociceptive and neuropathic pain co-exist.

- Nociceptive pain is the perception that follows activation of nociceptors by noxious stimuli but which is not associated with injury to peripheral nerves or the central nervous system. For clinical investigational purposes, nociceptive pain can be classified as somatic or visceral. Somatic pain is due to prolonged activation of the nociceptive receptors in somatic tissues, such as bone, joint, muscle or skin. In visceral pain, the visceral nociceptors are activated by different pathological mechanisms e.g. mechanical injury, inflammation, X-ray or toxic agents. These differences between visceral and somatic pain are not always clear in different pain models as several mechanisms can be involved (Bonica, 1992: xx-xxviii).

- The five main features of neuropathic pain are that the pain is of a burning, stabbing or shooting nature; it does not respond well to opioids; is mostly associated with a clinically

evident partial sensory deficit, thermal sensation usually being affected; the pain is often associated with autonomic instability and allodynia to touch, cold and movement occurs (Dubner, 1991:213).

New insight has been presented on standard drugs such as opioids, nonsteroidal anti-inflammatory drugs (NSAIDs) including selective cyclo-oxygenase-2 inhibitors and paracetamol. Traditional NSAIDs are widely prescribed as analgesics and anti-inflammatory agents because of their inhibition of prostanoid synthesis. However, major concerns regarding the safety profile of these drugs has resulted in considerable debate. The risks associated with traditional NSAIDs are well known and should be anticipated. Paracetamol is well established as an effective and very well tolerated agent in the management of mild to moderate pain. When Guindon (2007:2125) examined the concurrent use of paracetamol and an NSAID, evidence was found that the effect was superior to that of paracetamol used alone.

In a study conducted by Tacca *et al.* (2002:799-818), meloxicam demonstrated favourable tolerability profiles in large-scale comparative trials, where its gastro-intestinal tolerability was superior to that of nonselective NSAIDs.

1.1 Multimodal analgesia

Multimodal analgesia implies to a combination of different classes of analgesics as well as use of different sites of administration of the analgesics in order to improve pain relief. Secondly, it is hypothesised that when using multimodal analgesia, the adverse effects would be reduced because the adverse effect profiles of different analgesics differ (Kehlet, 1993:1048).

In a rat study conducted by Engelhardt *et al.* (1996:687), the analgesic and anti-exudative effect of meloxicam was enhanced in a super additive manner by the simultaneous administration of small doses of paracetamol. The increasing of efficacy of NSAIDs when used with paracetamol has previously been observed when taken with acetylsalicylic acid, flurbiprofen, indomethacin, diclofenac, naproxen, ketoprofen, fenoprofen, ibuprofen and suprofen. Pharmacological data in humans have confirmed the results of experiments for

these combinations. The experimental studies demonstrated the ability to improve analgesic efficacy by combining analgesics.

According to the guideline on Fixed-Combination Medicinal Products (1996:176) the potential advantages of a fixed combination included the following:

- i) An improvement in the benefit or risk assessment due to addition or potentiation of therapeutic activities of the substances and the counteracting by one substance of the adverse reaction produced by another one.
- ii) A simplification of therapy which improves patient compliance. When this is the only claim, it should be restricted to particular situations e.g. non-prescription products.

There are no analgesic agents specific for visceral or somatic pain. Also, neuropathic and nociceptive pain often responds differently to analgesics. The most currently used medicinal products, single or in combination, are opioid-like agonists, NSAIDs and paracetamol (Committee for Proprietary Medicinal Products, 2003). Paracetamol and NSAIDs are commonly used to treat abdominal cramping pain, e.g. pain associated with dysmenorrhoea, which is believed to be mediated mainly by excessive secretion of prostaglandins during the late luteal phase of the menstrual cycle (Budolf, 1987:453; Coco, 1999:489).

McQuay (2005:19-22) makes reference to meta-analysis demonstrating the analgesic superiority of a fixed-dose combination of tramadol and paracetamol treatment, over the individual components, without additional toxicity. Combination analgesic formulations are an important and effective means of pain relief. It is possible by combining low doses of opioids, where, when used in isolation are not very effective, with a non-opioid, to gain effective analgesic treatments.

This view is supported by Jung *et al.* (2004:1037-1045) because the administration of 30 mg of codeine in combination with aspirin is equivalent in analgesic effect to the administration of 65 mg of codeine. This combination has the advantage of reducing the amount of opioid required for pain relief and abolition of the pain via two distinct

mechanisms: inhibition of prostanoid synthesis and opioid inhibition of nociceptive transmission. Codeine phosphate, paracetamol and ibuprofen have also demonstrated rapid and effective analgesia for acute pain in postoperative dental pain.

According to Woodfork (2004:423-439), the non-opioid and NSAID analgesics act to decrease the generation of the mediators of pain at the site of tissue damage, although several of the drugs also have some effects within the central nervous system. The opioid analgesics are unique in that they not only block the incoming nociceptive signals to the brain but also act at higher brain centers, controlling the affective component of pain.

1.2 Objectives

The objective of this study was to develop a fixed dose combination product and determine the safety and intended dose regimen required for the relief of moderate to severe pain in adults. This fixed-dose combination product will contain paracetamol, codeine phosphate and meloxicam at doses where each active ingredient would be ineffective when used in isolation.

1.2.1 Effect on animals

To incorporate the patent application published by Norris (2005:1-6) on behalf of Adcock Ingram on the effects of a triple combination of meloxicam, a COX-2 selective inhibitor plus codeine phosphate, a weak opioid agonist and paracetamol, a centrally-acting COX-inhibitor and a NSAID peripherally acting analgesic, that was investigated in Sprague-Dawley rats. These agents were administered orally in a single dose to rats, at doses at which each of the individual ingredients in isolation are inactive. This was done to establish whether this combination had any antinociceptive activity in rats and whether the combination offers any synergy or super additive analgesia.

1.2.2 Pharmaceutical development

A fixed dose combination product with paracetamol, meloxicam and codeine phosphate will be developed based on the results obtained from the rat study.

The finished product will be tested according to in-house (Adcock Ingram) qualified and pharmacopoeial analytical methods to ascertain compliance to the set parameters. The formulation will be placed on stability as per International Conference on Harmonization (ICH) Guidelines where further testing will be conducted to establish the stability profile of the final product.

1.2.3 Clinical development

Two studies will be conducted to examine the pharmacokinetic properties, potential analgesic effect and safety of the fixed dose combination (FDC) capsule containing codeine phosphate, paracetamol and meloxicam in humans.

1.2.3.1 Pharmacokinetic Study

A pharmacokinetic study will be conducted to establish the bioavailability of the three drugs in a fixed dose combination product. This will be done by assessing their pharmacokinetics compared to those of products currently registered and marketed in South Africa with the same configuration of the active ingredients, where possible.

1.2.3.2 Proof-of-Concept/ Efficacy Study

A study to establish the safety and efficacy of the FDC capsules in females with primary dysmenorrhoea will be conducted. It is envisaged that the time to onset of action will be short given the rapid onset of action of paracetamol and codeine phosphate, while there should be enhancement of the therapeutic effect upon achieving maximum plasma meloxicam concentrations at a time when plasma paracetamol and codeine phosphate concentrations will be declining. Meloxicam's long elimination half-life, i.e. 20 hours, should contribute to a sustained "analgesic" effect, thereby extending the dose interval beyond 4 or even 6 hours. It is postulated that the combination of meloxicam, a NSAID of the oxicam class, with paracetamol, will act synergistically in providing relief of pain associated with dysmenorrhoea, and that the addition of codeine phosphate will further enhance this synergy.

CHAPTER 2

ANALYTICAL AND BIO-ANALYTICAL TESTING METHODS

INTRODUCTION

The analytical methods used for testing the capsules were developed and qualified (characterised) at Adcock Ingram Limited, Research and Development and the Bioanalytical methods were developed and validated at Farmovs-Parexel Bioanalytical Services Division, in Bloemfontein, South Africa.

Analytical methods

2.1 Physicals

2.1.1 Description

Examine the sample visually and describe with respect to the following:

Dosage form

Colour of capsule cap and body

Capsule contents

Odour of capsule contents

2.1.2 Disintegration

Place one capsule into each of the six baskets. Operate the apparatus without discs, using water maintained at 37 °C, as the immersion fluid. Operate the apparatus until all six capsules have disintegrated. Record the time of complete disintegration, where complete disintegration is defined as that state in which any residue of the unit, except fragments of capsule shell, remaining on the screen of the test apparatus, is a soft mass having no palpably firm core (BP, 2007).

2.1.3 Average fill mass (AFM)

Weigh an intact capsule. Open the capsule without losing any part of the shell and remove the contents as completely as possible. Weigh the empty shell. The weight of the contents is the difference between the weighings. Repeat the procedure with a further 19 capsules selected at random. Determine the average weight (BP, 2007).

2.1.4 Uniformity of mass

Use the individual recorded results obtained from average fill mass test. Not more than (NMT) 2 capsules in 20 deviate by more than 7.5 % of the average capsule fill mass. No capsule deviates by more than 15 % of the average capsule fill mass (BP, 2007).

2.1.5 Loss on drying

Accurately weigh approximately 2.0 g of capsule contents and dry over phosphorous pentoxide at a pressure of 1.5 kPa to 2.5 kPa, at room temperature for 4 hours. NMT 5 % loss (BP, 2007).

2.2 Assay of codeine phosphate, paracetamol and 4-aminophenol

Paracetamol label claim: 250 mg/capsule
Codeine phosphate label claim: 10 mg/capsule
4-aminophenol: NMT 0.1 % m/m of paracetamol content

2.2.1 Analytical reagents

The analytical reagents, grade and the suppliers used for the assay are given in Table 2.1 below.

Table 2.1: Analytical reagents used for the assay of paracetamol, codeine phosphate and 4-aminophenol

Reagent	Grade	Supplier
Milli-Q (Water)	HPLC	Microsep system-Academic
Trifluoroacetic acid	HPLC grade	Fluka
Acetonitrile	HPLC grade	Sigma Aldrich or Burdick & Jackson
Ammonia solution	AR grade	Sigma Aldrich
Tetrahydrofuran (THF)	HPLC grade	Sigma Aldrich
Paracetamol	Primary standard	Sigma
Codeine phosphate	Secondary standard	Fine Chemicals
4-aminophenol	Primary standard	Sigma

Mobile phase preparation (Degas before use)

Buffer preparation

Dilute 1 ml of trifluoroacetic acid in 1 000 ml of water. Adjust pH to 3.0 ± 0.05 with 6M ammonia.

Pre-mixed: Mobile phase

Mix 880 ml buffer solution with 120 ml acetonitrile.

For on line solvent mixing instruments

Mobile phase A: Buffer solution

Mobile phase B: Acetonitrile

Solvent: Water

Standard preparation (for initial testing) Solution A

Accurately weigh 50 mg codeine phosphate standard into a 100 ml volumetric flask. Dissolve in and dilute to volume with solvent.

Accurately weigh 250 mg of paracetamol standard into a 200 ml volumetric flask. Add 20 ml of acetonitrile to dissolve. Pipette in 20 ml of Solution A and dilute to volume with the solvent.

Standard preparation (for stability purposes) Solution A

Accurately weigh 50 mg of 4-aminophenol standard into a 100 ml volumetric flask, add 20 ml acetonitrile and 5 ml of the solvent to dissolve. Dilute to volume with acetonitrile. Pipette 5 ml of this solution into a 100 ml volumetric flask and dilute to volume with acetonitrile.

Accurately weigh 50 mg of codeine phosphate standard into a 100 ml volumetric flask. Dissolve and dilute to volume with the solvent (Solution B).

Accurately weigh 250 mg of paracetamol standard into a 200 ml volumetric flask. Add 10 ml acetonitrile to dissolve. Pipette 10 ml of Solution A and 20 ml of Solution B into the flask and dilute to volume with solvent.

Sample preparation

Weigh and powder the contents of 20 capsules, using a pestle and mortar. Accurately weigh capsule powder equivalent to one capsule mass (± 400 mg) into a 200 ml volumetric flask. Add 20 ml of acetonitrile and 100 ml of solvent and ultrasonicate for 10 minutes. Cool and dilute to volume with solvent.

2.2.2 HPLC procedure

Filter both standard and sample preparations through a non-sterile 33 mm Millex-HV Hydrophillic Durapore® (PVDF) 0.5 μ m syringe filter unit, from Millipore before injecting onto the column. Analyse according to the High performance liquid chromatography (HPLC) conditions given in Table 2.2.

2.2.3 Calculation for software users

Standard calibration: **Paracetamol**

$$\frac{\text{Standard mass (mg)} \times \% \text{ purity}}{100} \approx 250 \text{ mg}$$

Standard calibration: **Codeine phosphate**

$$\frac{\text{Standard mass (mg)} \times \% \text{ purity}}{5 \times 100} \approx 10 \text{ mg}$$

Sample calculation

$$\frac{\text{HPLC result} \times \text{average capsule fill mass (mg)}}{\text{Sample mass (mg)}} = \text{mg/capsule}$$

Degradation calculation: **4-aminophenol**

$$\frac{A_u \times \text{standard mass (mg)} \times \% \text{ purity} \times 5 \times 10 \times 200 \times \text{average capsule fill mass (mg)} \times 100}{A_s \times 100 \times 100 \times 100 \times 200 \times \text{sample mass (mg)} \times \text{paracetamol assay (mg/capsule)}} \\ = \% \text{ m/m of the paracetamol content}$$

Where: A_u = Peak area of 4-aminophenol in sample chromatogram

A_s = Peak area of 4-aminophenol in standard chromatogram

Table 2.2: HPLC chromatographic conditions for paracetamol, codeine phosphate and 4-aminophenol

Analytical Instrument	HPLC equipment, Waters, 2487 dual λ absorbance detector / PDA 2996 detector. Waters 2695 Alliance Separations Module and Waters Empower Pro software.
System suitability	Inject the standard solution first to verify that the system is suitable before proceeding with the assay.
RSD	NMT 2.0 % of six injections on standard solution
Tailing factor	NMT 2.0
Column efficiency	NLT 2 500 theoretical plates (USP tangent method) for paracetamol peak NLT 4.0 between paracetamol and 4-aminophenol (stability only)
Column	Atlantis [®] T3 (4.6 x 75) mm, Waters Particle size : 5 μ m Particle shape : Spherical Pore size : 100 Å Carbon load : 12.69 % End-capped : Yes pH range : 2.0 to 7.0
Temperature	15 °C to 25 °C
Mobile phase	Mobile phase A (88): Mobile phase B (12)
Wavelength	275 nm for paracetamol and 4-aminophenol and 240 nm for codeine phosphate
Injection volume	5 μ l for paracetamol and codeine phosphate and 10 μ l for 4-aminophenol
Flow rate	1.0 ml/minute
Retention times (approximately)	4-aminophenol: 1.3 minutes Paracetamol: 5.04 minutes Codeine phosphate: 2.3 minutes
Run time (approximately)	6 minutes

2.3 Assay of meloxicam

Meloxicam label claim: 2.5 mg/capsule

Benzothiazine ethyl ester: NMT 0.5 % m/m of the meloxicam content

2-amino-5-methylthiazole: NMT 0.5 % m/m of the meloxicam content

2.3.1 Analytical reagents

The analytical reagents used, grade and the suppliers used are given in Table 2.3.

Table 2.3: Analytical reagents used for the assay of meloxicam

Reagent	Grade	Supplier
Water	Milli-Q water	Microsep System-Academic
Ammonium acetate	HPLC grade	Fluka
Acetonitrile	HPLC grade	Sigma-Aldrich or Burdick & Jackson
Formic acid concentrated	AR grade	Fluka
Tetrahydrofuran (THF)	HPLC grade	Sigma-Aldrich
Meloxicam	Secondary standard	Denvados Quimicos S.A.
Benzothiazine ethyl ester	Primary standard	Industrial Analytical
2-amino-5-methylthiazole	Primary standard	Industrial Analytical

Mobile phase preparation (Degas before use)

Buffer preparation

Dissolve 1.54 g of ammonium acetate in 1 000 ml of water. Adjust pH to 4.5 $\pm$ 0.05 with formic acid.

Pre-mixed

Mobile phase: Meloxicam assay

Mix 700 ml of the buffer solution with 300 ml of acetonitrile.

Mobile phase – Line A (Meloxicam degradation products)

Mix 950 ml of the buffer solution with 50 ml of acetonitrile.

Mobile phase – Line B (Meloxicam degradation products)

Mix 400 ml of the buffer solution with 600 ml of acetonitrile.

For on line solvent mixing instruments

Mobile phase A: Buffer solution

Mobile phase B: Acetonitrile

Solvent

Water (90): Acetonitrile (10)

Standard preparation (initial testing and content uniformity) Solution A

Accurately weigh 50 mg of meloxicam standard into a 100 ml volumetric flask, dissolve in 60 ml of tetrahydrofuran and dilute to volume with the solvent. Pipette 5 ml of Solution A into a 50 ml volumetric flask and dilute to volume with the solvent.

Standard preparation (for stability purposes) Solution A

Accurately weigh 5 mg of benzothiazine ethyl ester and 5 mg of 2-amino-5-methylthiazole standards into a 100 ml volumetric flask, dissolve in and dilute to volume with the solvent.

Accurately weigh 50 mg of meloxicam standard into a 100 ml volumetric flask, dissolve in 60 ml of tetrahydrofuran and dilute to volume with the solvent (Solution B). Pipette in 5 ml of Solution A and 5 ml of Solution B into a 50 ml volumetric flask. Dissolve in and dilute to volume with the solvent.

Sample preparation (assay purposes)

Weigh and powder the contents of 20 capsules, using a pestle and mortar. Accurately weigh capsule powder equivalent to one capsule mass into a 50 ml volumetric flask. Add 20 ml of solvent and 10 ml of tetrahydrofuran and ultrasonicate for 15 minutes. Cool and dilute to volume with the solvent.

Sample preparation (content uniformity purposes)

Transfer one whole capsule into a 50 ml volumetric flask. Add 20 ml of the solvent and 10 ml of tetrahydrofuran; ultrasonicate for 15 minutes. Cool and dilute to volume with the solvent.

2.3.2 HPLC procedure

Filter both standard and sample preparations through a non-sterile 33 mm Millex-HV Hydrophilic Durapore® (PVDF) 0.45 µm syringe filter unit, from Millipore before injecting onto the column. Analyse according to the HPLC conditions given in Tables 2.4 and 2.5.

2.3.3 Calculation for software users

Meloxicam (assay purposes)

$$\frac{\text{Standard mass (mg)} \times \% \text{ purity}}{20 \times 100} \approx 2.5 \text{ mg}$$

Meloxicam (content uniformity purposes)

$$\frac{\text{Standard mass (mg)} \times 2 \times \% \text{ purity}}{100} \approx 100 \%$$

Sample calculation (assay purposes)

$$\frac{\text{HPLC result} \times \text{average capsule fill mass (mg)}}{\text{Sample mass (mg)}} = \text{mg/capsule}$$

Sample calculation (content uniformity purposes)

$$\text{HPLC result} = \% \text{ meloxicam}$$

Degradation calculation: Benzothiazine ethyl ester and 2-amino-5-methylthiazole

$$\frac{\text{Au} \times \text{standard mass (mg)} \times \% \text{ purity} \times 5 \times 5 \times 50 \times \text{average capsule fill mass (mg)} \times 100}{\text{As} \times 100 \times 100 \times 100 \times 50 \times \text{sample mass (mg)} \times \text{meloxicam assay (mg/capsule)}} = \% \text{ m/m of the meloxicam content}$$

Where: Au = Peak area in sample chromatogram
As = Peak area in standard chromatogram

Table 2.4: HPLC chromatographic conditions for meloxicam assay

Analytical instrument	HPLC equipment, Waters 2487 dual λ absorbance detector / PDA 2996 detector Waters 2695 Alliance Separations Module and Waters Empower Pro software.
System suitability	Inject the standard solution first to verify that the system is suitable before proceeding with the assay.
RSD	NMT 2.0 % of six injections on standard solution
Tailing factor	NMT 2.0
Column efficiency	NLT 1 000 theoretical plates (USP tangent method) for meloxicam
Column	XBridge™ C18 (4.6 x 75) mm, Waters Particle size : 3.5 μ m Particle shape : Spherical Pore size : 135 Å Carbon load : 17.5 % End-capped : Yes pH range : 2.0 to 8.0
Temperature range	15 °C to 25 °C
Mobile phase	Mobile phase A (70): Mobile phase B (30)
Wavelength	362 nm for meloxicam
Injection volume	5 μ l
Flow rate	1.0 ml/minute
Retention times (approximately)	Meloxicam : 3.8 minutes
Run time (approximately)	5 minutes

Table 2.5: HPLC chromatographic conditions for meloxicam degradation products

Analytical instrument	HPLC equipment, Waters 2487 dual λ absorbance detector / PDA 2996 detector Waters 2695 Alliance Separations Module and Waters Empower Pro software.
System suitability	Inject the standard solution first to verify that the system is suitable before proceeding with the assay.
RSD	NMT 2.0 % of six injections on standard solution
Tailing factor	NMT 2.0
Column efficiency	NLT 1 000 theoretical plates (USP tangent method) for meloxicam peak NLT 3.0 between benzothiazine ethyl ester and meloxicam peak at 315 nm (stability only)
Column	XBridge™ C18 (4.6 x 75) mm Particle size : 3.5 μ m Particle shape : Spherical Pore size : 135 Å Carbon load : 17.5 % End-capped : Yes pH range : 2.0 to 8.0
Mobile phase	The gradient on-line solvent mixing and the premix solvent are shown in Tables 2.6 and 2.7.
Wavelength	254 nm for 2-amino-5-methylthiazole 315 nm for benzothiazine ethyl ester and meloxicam
Injection volume	5 μ l
Flow rate	1.0 ml/minute
Retention times (approximately)	2-amino-5-methylthiazole: 1.6 minutes Benzothiazine ethyl ester : 6.4 minutes
Run time (approximately)	9 minutes

Table 2.6: Gradient table – On line solvent mixing

	Time (minutes)	Flow rate (ml/minute)	% A	% B	*Curve
1.	0.00	1.00	95	5	6
2.	2.50	1.00	95	5	6
3.	3.50	1.00	40	60	6
4.	7.00	1.00	40	60	6
5.	7.10	1.00	95	5	6
6.	9.00	1.00	95	5	6

Table 2.7: Gradient table – Premixed solvent

	Time (minutes)	Flow rate (ml/minute)	% A	% B	*Curve
1.	0.00	1.00	100	0	6
2.	2.50	1.00	100	0	6
3.	3.50	1.00	0	100	6
4.	7.00	1.00	0	100	6
5.	7.10	1.00	100	0	6
6.	9.00	1.00	100	0	6

* Code 6 means linear change

2.4 Dissolution for paracetamol and codeine phosphate

Paracetamol label claim: 250 mg/capsule

Codeine phosphate label claim: 10 mg/capsule

2.4.1 Analytical reagents

The analytical reagents, grade and the suppliers used are given in Table 2.8.

Table 2.8: Analytical reagents for paracetamol and codeine phosphate dissolution

Reagent	Grade	Supplier
Water	Milli-Q water	Microsep System-Academic
Trifluoroacetic acid	HPLC grade	Fluka
Hydrochloric acid (32 %)	AR grade	Merck
Ammonia solution	AR grade	Sigma Aldrich
Acetonitrile	HPLC grade	Sigma Aldrich or Burdick & Jackson
Paracetamol	Secondary standard	Fine chemicals
Codeine phosphate	Secondary standard	Fine Chemicals

Preparation of 0.01 HCl Dissolution medium

Add 7.9 ml hydrochloric acid 32 % to 8 liters of purified water and mix. Heat to approximately 37 °C

2.4.2 Dissolution conditions (in-house)

Apparatus: USP paddle assembly

Medium: 900 ml of 0.01N HCl, preheated and maintained at 37 °C

Paddle speed: 50 rpm

Sampling (automated): Dissoette II or Autoplus Maximizer

Filters (standard solution): Non-sterile 33 mm Millex-HV Hydrophilic Durapore® (PVDF), 0.45 µm syringe filter unit, from Millipore.

Filters (test solution): Hanson Research online sample filters 10 µm P/N 27-101-074 (Dissoette)

Hanson Research online sample filters 10 µm P/N 27-101-083 (Maximizer)

Withdrawal times: 10, 15 and 30 minutes

Tolerance: NLT 80 % (Q) in 30 minutes

2.4.2.1 Dissolution procedure

Place one capsule into each of the six dissolution vessels taking care to exclude air bubbles from the surface of the capsules. Immediately operate the apparatus rotating the

paddles at 50 revolutions per minute (rpm). Withdraw the samples from a zone midway between the surface of the medium and the top of the rotating paddles, and not less than 1 cm from the wall of the vessel at the specified withdrawal times. Place samples into vials that have been labeled accordingly and analyse under the HPLC chromatographic conditions given in Table 2.9.

Mobile phase preparation (Degas before use)

Buffer solution preparation

Dilute 1.0 ml of trifluoroacetic acid in 1 000 ml of water. Adjust pH to 3.0 ± 0.05 with ammonia acid.

Pre-mixed

Mobile phase

Mix 880 ml buffer solution with 120 ml of acetonitrile.

For online solvent mixing instruments

Mobile phase A: Buffer solution

Mobile phase B: Acetonitrile

Standard preparation (Solution A)

Accurately weigh 56 mg of codeine phosphate into a 50 ml volumetric flask. Add 5 ml of the dissolution medium and shake to dissolve. Dilute to volume with the dissolution medium. Accurately weigh 278 mg of paracetamol standard into a 100 ml volumetric flask, add 10 ml of acetonitrile and 20 ml of the dissolution medium to dissolve. Pipette in 10 ml of Solution A and dilute to volume with dissolution medium (Solution B). Pipette 10 ml of solution B into a 100 ml volumetric flask and dilute to 100 ml with the dissolution medium.

2.4.3 HPLC procedure

Filter the standard preparation through a non-sterile 33 mm Millex-HV Hydrophillic Durapore® (PVDF) 0.45 µm syringe filter unit, from Millipore and inject into the HPLC column, using the chromatographic conditions given in Table 2.9.

2.4.4 Calculation for software users

Standard calibration: **Paracetamol**

$$\frac{\text{Standard mass (mg)} \times \% \text{ purity} \times 9}{25 \times 100} \approx 100 \%$$

Standard calibration: **Codeine phosphate**

$$\frac{\text{Standard mass (mg)} \times \% \text{ purity} \times 9}{5 \times 100} \approx 100 \%$$

Sample calculation

HPLC result = % active dissolved

Table 2.9: HPLC chromatographic conditions for paracetamol and codeine phosphate dissolution

Analytical instrument	HPLC equipment, Waters 2487 dual λ absorbance detector / PDA 2996 detector Waters 2695 Alliance Separations Module and Waters Empower Pro software.
System suitability	Inject the standard solution first to verify that the system is suitable before proceeding with the assay.
RSD	NMT 2.0 % of six injections on standard solution
Tailing factor	NMT 2.0
Column efficiency	NLT 2 000 theoretical plates (USP tangent method) for paracetamol peak NLT 4.0 between paracetamol and 4-aminophenol (stability)
Column	Atlantis® T3 (4.6 x 75) mm, Waters Particle size : 5 μ m Particle shape : Spherical Pore size : 100 Å Carbon load : 12.69 % End-capped : Yes pH range : 2.0 to 7.0

Table 2.9: Continued

Temperature	15 °C to 25 °C
Mobile phase	Mobile phase A (88): Mobile phase B (12)
Wavelength	275 nm for paracetamol and 4-aminophenol 240 nm for codeine phosphate
Injection volume	20 µl
Flow rate	1.0 ml/minute
Retention times	Paracetamol: 2.3 minutes
(approximately)	Codeine phosphate: 3.9 minutes
Run time	6 minutes
(approximately)	

2.5 Dissolution for meloxicam

Meloxicam label claim: 2.5 mg/capsule

2.5.1 Analytical reagents

The analytical reagents, grade and the suppliers used are given in Table 2.10.

Table 2.10: Analytical reagents for meloxicam dissolution

Reagent	Grade	Supplier
Water	Milli-Q water	Microsep System-Academic
Ammonium acetate	HPLC grade	Fluka
Acetonitrile	HPLC grade	Sigma Aldrich or Burdick & Jackson
Formic acid concentrated	AR grade	Fluka
Monobasic sodium phosphate monohydrate	AR grade	Merck
Dibasic sodium phosphate dehydrate	AR grade	Merck

Table 2.10: Continued

Reagent	Grade	Supplier
Sodium lauryl sulphate	AR grade	Fluka
Sodium hydroxide Pellets	AR grade	Merck
Meloxicam	Secondary standard	Denvados Quimicos S.A.

Preparation of Dissolution medium (Phosphate buffer pH 6.8 with 0.1 % SLS)

Accurately weigh and transfer 45 g of monobasic sodium phosphate monohydrate, 28 g of dibasic sodium phosphate and 8 g of sodium lauryl sulphate (SLS) into a stainless steel vessel containing 6 liters of purified water. Add 4 g of sodium hydroxide pellets and adjust the pH to 6.8 with 1M sodium hydroxide pellets. Make up to 8 liters with purified water.

2.5.2 Dissolution conditions (in-house)

Apparatus: USP paddle assembly

Medium: 900 ml of phosphate buffer pH 6.8 with 0.1 % SLS, preheated and maintained at 37 °C

Paddle speed: 50 rpm

Sampling (automated): Autoplus Maximizer

Filters (standard solution): Non-sterile 33 mm Millex-HV Hydrophillic Durapore® (PVDF), 0.45 µm syringe filter unit, from Millipore

Filters (test solution): Hanson Research online sample filters 10 µm P/N 27-101-083 (Maximizer)

Withdrawal times: 30, 45 and 60 minutes

Tolerance: NLT 80 % (Q) in 60 minutes

2.5.3 Dissolution procedure

Place one capsule into each of the six dissolution vessels taking care to exclude air bubbles from the surface of the capsule. Immediately operate the apparatus rotating the paddles at 50 rpm. Withdraw the samples from a zone midway between the surface of the medium and the top of the rotating paddles, and not less than 1 cm from the wall of the vessel at the specified withdrawal times. Place samples into the vials that have been

labeled accordingly. Analyse under the HPLC chromatographic conditions given in Table 2.11.

Mobile phase preparation (Degas before use)

Buffer solution preparation

Dissolve 1.54 g of ammonium acetate in 1 000 ml of water. Adjust pH to 4.5 ± 0.05 with formic acid.

Pre-mixed

Mobile Phase

Mix 700 ml of the buffer solution with 300 ml of acetonitrile.

For on line solvent mixing instruments

Mobile phase A: Buffer solution

Mobile phase B: Acetonitrile

Standard preparation (Solution A)

Accurately weigh 56 mg of meloxicam into a 100 ml volumetric flask. Dissolve in 60 ml of tetrahydrofuran and dilute to volume with water. Dilute 10 ml of this solution to 100 ml with the dissolution medium.

2.5.4 HPLC procedure

Filter the standard preparation through a non-sterile 33 mm Millex-HV Hydrophillic Durapore® (PVDF) 0.45 µm syringe filter unit, from Millipore and inject into the HPLC column, using HPLC conditions given in Table 2.11.

2.5.5 Calculation for software users

Standard calibration: **Meloxicam**

$$\frac{\text{Standard mass (mg)} \times \% \text{ purity} \times 9}{100 \times 5} \approx 100 \%$$

Sample calculation

HPLC result = % dissolved

Table 2.11: HPLC chromatographic conditions for meloxicam dissolution

Analytical instrument	HPLC equipment, Waters 2487 dual λ absorbance detector / PDA 2996 detector Waters 2695 Alliance Separations Module and Waters Empower Pro software.
System suitability	Inject the standard solution first to verify that the system is suitable before proceeding with the assay.
RSD	NMT 2.0 % of six injections on standard solution
Tailing factor	NMT 2.0
Column efficiency	NLT 1 000 theoretical plates (USP tangent method) for meloxicam peak
Column	XBridge™ C18 (4.6 x 75) mm, Waters
	Particle size : 3.5 μ m
	Particle shape : Spherical
	Pore size : 135 Å
	Carbon load : 17.5 %
	End-capped : Yes
	pH range : 2.0 to 8.0
Temperature	15 °C to 25 °C
Mobile phase	Mobile phase A (70): Mobile phase B (30)
Wavelength	362 nm
Injection volume	40 μ l
Flow rate	1.0 ml/minute
Retention times	Meloxicam : 3.9 minutes (approximately)
Run time	5 minutes (approximately)

2.6 Qualification of the analytical methods

According to Boundreau *et al.* (2004:54-66), limiting early method validation to the essential elements helps to maintain flexibility during the very fluid stages of early product development. The requirements for method validation are clear for new drug applications and many other worldwide marketing applications. These documents are specified in

documents from the International Conference on Harmonisation (ICH), regulatory authorities and pharmacopeias.

However, Boundreau *et al.* (2004:54-66) argued that the validation guidelines application to early drug development phases are not specific, thus a phased validation of product development is carried out. Some of the experiments or tests are delayed until later in the product development stage, e.g. Intermediate precision: during early development, when methods are typically operated in one laboratory and by a few analysts, it is not necessary to determine the intermediate precision of an assay method. By phasing the methods validation activities, resources can be optimised while maintaining a good scientific approach to a pharmaceutical development. Ritter (2004:2-9) added that typically methods are only validated during Phase III studies in preparation for conducting conforming validation studies and for submitting a marketing application.

Due to the above discussions, it was viewed not necessary to conduct a full validation of the analytical methods at this development stage, since the developed product is going to be used for Phase I and early Phase II trials. Dissolution was also not validated nor qualified since it was deemed not yet necessary to ensure batch-to-batch consistency at this early development phase. Only one batch for each product was manufactured for clinical trial use and short term stability assessment.

During qualification process, the following parameters were assessed:

2.6.1 Qualification (mini-validation) of the assay method for paracetamol, codeine phosphate and 4-aminophenol

2.6.1.1 Selectivity

The placebo (sample without analyte) was prepared in the same way as the sample and determined under the proposed chromatographic conditions in the analytical procedure and injected.

The degradation product for paracetamol; 4-aminophenol was determined using the described method. Their specified limit was set at NMT 0.1 % of the paracetamol content.

No significant peaks at the retention time of the analyte peaks were observed as shown in Appendix B. This shows that the excipients do not interfere with the analyte peaks. The peaks of interest were found to be pure. The results obtained show that the developed method is selective for the determination of paracetamol, codeine phosphate and the 4-aminophenol in the capsule formulation.

2.6.1.2 Linearity and range

Linearity and range (paracetamol and codeine phosphate)

The linearity of the standard response was determined by injecting each standard twice. The range for paracetamol and codeine phosphate was at seven concentration levels, ranging from 10 to 150 %.

Linearity and range (4-aminophenol)

The range for 4-aminophenol was at concentration levels from 10 to 125 %.

Results

The results in Table 2.12 show that there is excellent correlation between the peak area and the concentrations of paracetamol, codeine phosphate and 4-aminophenol within their respective concentration ranges. The linearity plots are shown in Appendix A.

The slope on the regression was developed from the mathematical transformation of the response data. The acceptance criterion for correlation and deviation from the y-intercept was met. The response is linear and linear regression has an intercept not significantly different from zero and a correlation coefficient close to 1.0.

Table 2.12: Summary of the linearity results for paracetamol, codeine phosphate and 4-aminophenol

Parameter	Acceptance criteria	Paracetamol	Codeine phosphate	4-aminophenol
Linearity	Correction co-efficient NLT 0.999	0.999981	0.999974	0.9999654

2.6.1.3 Accuracy

The accuracy of the methods for the actives were evaluated by applying the method to a placebo to which known amounts of paracetamol and codeine phosphate corresponding to 90 %, 100 % and 110 % of the label claim were spiked. Each preparation was injected three times. Accuracy was calculated as the percentage of the analyte recovered from the formulation matrix. The mean percentage recovered for paracetamol and codeine phosphate is shown in Table 2.13 and the results indicate good accuracy.

2.6.1.4 Precision

Precision of the method was tested by injecting each of the six samples against the 100 % standard and the RSD and mean values were calculated. The results in Table 2.13 show that the obtained RSD values were within the specified limits. This indicates that the system shows good reproducibility of the method.

2.6.1.5 System suitability

System suitability was demonstrated throughout the phase validation. The resolution between paracetamol and 4-aminophenol was 8.50. This indicated good resolution as the limit was set as NLT 4.0. The results obtained show that the method complies with the requirements for system suitability.

Table 2.13: System suitability, precision and accuracy of the paracetamol and codeine phosphate method

Parameter	Acceptance criteria	Paracetamol	Codeine phosphate
System suitability			
% RSD	NMT 2.0 %	0.166	0.161
Tailing factor	NMT 2.0 %	1.08	1.07
Theoretical plates (USP tangent)	Paracetamol: NLT 2 500 Codeine phosphate: NLT 1 000	5094	2935
Precision	% RSD: NMT 2 %	0.641 %	0.720 %

Table 2.13: Continued

Parameter	Acceptance criteria	Paracetamol	Codeine phosphate
Accuracy			
90 %	98 – 102 %	99.73 %	99.53 %
		99.65 %	99.49 %
		100.35 %	100.03 %
100 %	98 – 102 %	99.97 %	99.19 %
		100.28 %	99.49 %
		100.22 %	99.83 %
110 %	98 – 102 %	99.95 %	99.86 %
		100.18 %	100.27 %
		99.89 %	100.02 %
% Average	98 – 102 %	100.02 %	99.75 %
Average %	NMT 2 %	0.235 %	0.278 %
RSD			

2.6.2 Qualification of the assay method for meloxicam and its degradation products

2.6.2.1 Specificity

The placebo was prepared in the same way as the sample, under the conditions described in the analytical procedure and was injected.

The degradation products, 2-amino-5-methylthiazole and benzothiazine ethyl ester were determined by using the described method. Their specified limit is NMT 0.5 % of the meloxicam content.

No significant peaks at the retention time of the analyte peaks were observed as shown in Appendix B. This shows that the excipients do not interfere with the analyte peaks. The peaks of interest were found to be pure. The results obtained show that the developed method is selective for the determination of meloxicam and its degradation products in the capsule formulation.

2.6.2.2 Linearity and range

Linearity and range (meloxicam)

The linearity of the standard response was determined by injecting each standard twice.

The range for meloxicam was at seven concentration levels, 10 to 150 %.

Linearity and range (2-amino-5-methylthiazole and benzothiazine ethyl ester)

The range for benzothiazine ethyl ester and 2-amino-5-methylthiazole was at five concentration levels, from 10 to 150 %.

Results

The results in Table 2.14 show that there is excellent correlation between the peak area and concentrations meloxicam and benzothiazine ethyl ester and 2-amino-5-methylthiazole within their respective concentration ranges. The linearity plots are shown in Appendix A. The slope on the regression was developed from the mathematical transformation of the response data. The acceptance criterion for correlation and deviation from the y-intercept was met. The response is linear and linear regression has an intercept not significantly different from zero and a correlation coefficient close to 1.0.

Table 2.14: Linearity and range results for meloxicam and its degradation products

Parameter	Acceptance criteria	Meloxicam	Benzothiazine ethyl ester	2-amino-5-methylthiazole
Linearity	Correction co-efficient NLT 0.999	0.999940	0.999826	0.999901

2.6.2.3 Accuracy

The accuracy of the methods for the meloxicam was evaluated by applying the method to a placebo to which known amounts of meloxicam corresponding to 90 %, 100 % and 110 % of the label claim was spiked. Each preparation was injected three times. Accuracy was calculated as the percentage of the analyte recovered from the formulation matrix. The mean percentage recovered for meloxicam is shown in Table 2.15 and the results indicate

good accuracy.

2.6.2.4 Precision

Precision of the method was tested by injecting each of the six samples against the 100 % standard and the RSD and mean values were calculated. The results shown in Table 2.15 show that the obtained RSD values were within the specified limits. This indicates that the system shows good reproducibility of the method.

2.6.2.5 System suitability

System suitability was demonstrated throughout the phase validation. The resolution between meloxicam and benzothiazine ethyl ester is 8.95. This showed good resolution as the limit was set as NLT 3.0. The results in Table 2.15 show that the method complies with the requirements for system suitability.

Table 2.15: Summary of the system suitability, precision and accuracy for meloxicam

Parameter	Acceptance criteria	Results
System suitability	RSD: NMT 2.0 %	0.32
	Tailing factor : NMT 2.0 %	1.02
	Theoretical plates (USP tangent): NLT 1 000	2 180
Precision	RSD: NMT 2.0 %	0.423 %
Accuracy	% Relative recovery	100.61 %
		100.51 %
		100.05 %
		100.84 %
100 %	98 – 102 %	101.03 %
		100.72 %
110 %	98 – 102 %	100.92 %
		101.64 %
		100.61 %

Table 2.15 Continued

Parameter	Acceptance criteria	Results
% Average	98 – 102 %	100.77 %
Average % RSD	NMT 2 %	0.326 %

2.7 Summary and Conclusion

2.7.1 Summary

The results obtained from this study show that the HPLC methods used to test the actives and degradation products in the capsules give:

- Linear results in the respective range of analyte in the test solution
- Specific, accurate and precise results in the range 90 to 110 % of the labeled amount of the active ingredients.

2.7.2 Conclusion

The development of this method provided an acceptable degree of precision and selectivity for the simultaneous determination of the paracetamol, codeine phosphate and 4-aminophenol; and for meloxicam, benzothiazine ethyl ester and 2-amino-5-methylthiazole. It is therefore concluded that the method is suitable for its intended purposes of testing paracetamol, codeine phosphate, meloxicam and the degradation products during this early phase of product development, Phase I and early Phase II clinical trials.

2.8 Thermal methods

2.8.1 Differential Scanning Calorimetry

DSC was used to investigate the changes in the energy flow in samples when exposed to increase temperature. The results were used to identify possible polymorphs and to characterise the different forms in view of their melting points.

DSC thermograms of the samples were obtained using the Shimadzu DSC-50 instrument (Shimadzu, Kyoto, Japan). 3 - 5 mg of the samples were weighed into aluminium pans with pierced crimped on lids. The samples were heated at a constant heating rate of 10 °C/min under nitrogen purge with a flow rate of 35 ml/min.

Sample preparation

The ingredients were first mixed in a ratio of 1:1 in different combinations. The procedure described above was then followed on the Shimadzu.

2.9 Sartorius Moisture Analyser (for in-process control)

Spread two grams of the granules evenly on the aluminium tray. Set the moisture analyser parameter for 20 minutes at 60 °C.

2.10 Bio-analytical methods

The Bio-analytical study was performed at Farmovs-Parexel Bioanalytical Services Division, on behalf of Adcock Ingram Limited.

The assay methods used were validated in accordance with the Farmovs-Parexel Bioanalytical Services Division standard operating procedures (SOP) and acceptance criteria current at that time.

Storage conditions

The original and duplicate sets of sample aliquots were stored below –20 °C in separate freezers at the Farmovs-Parexel Bioanalytical Services Division from the date of receipt until the date of analysis.

GLOSSARY OF ABBREVIATIONS

% Dev (% Deviation) The difference between the true nominal value and the
Synonymous to: % Bias value obtained, expressed as a percentage.

$$\% \text{ Dev (or \% Bias)} = \left(\frac{\text{Found value} - \text{Nom value}}{\text{Nom value}} \right) \times 100$$

% nom The closeness of the agreement between the result of a
measurement and the true (nominal) value of the
measured expressed as percentage.

$$\% \text{ Nom} = \left(\frac{\text{Found value}}{\text{Nominal value}} \right) \times 100$$

~ Approximately

BA	Bioanalytical
BASD	Bioanalytical Services Division
BLQ	Below the lower limit of quantification
C	Celsius
C _{max}	Maximum plasma concentration
CV	Coefficient of variation
EDTA	Ethylenediaminetetraacetic acid
GCP	Good Clinical Practice
ISTD	Internal Standard
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry
LLOQ	Lower Limit of Quantification
MNR-ESD outlier test	The Maximum Normal Residual test modified by using a backwards elimination algorithm referred to as the Extreme Studentised Deviate in order to be able to detect multiple outliers. Hawkins (Hawkins, D.M., Identification of Outliers, Chapman and Hall, 1980) has shown this to be an acceptable method for detecting multiple outliers, as the error is well defined and acceptably small.

2.10.1 Determination of paracetamol in human plasma by LC-MS/MS

2.10.1.1 Quantification of study samples

The quantification of study samples was carried out using the assay method described in this section. During validation, this assay method provided an acceptable degree of accuracy and precision over the concentration range 102.01 – 13055 ng/ml based on peak area ratios with the Wagner calibration curves ($\ln(y) = a(\ln(x))^2 + b(\ln(x)) + c$).

The analytical reagents, grade, supplier and reference numbers used are given in Table 2.16. The water used was purified by Millipore Elix 10 reverse osmosis and Milli-Q® (Millipore) Gradient A10 polishing system.

Table 2.16: Analytical reagents used in the assay of paracetamol

Reagent	Grade	Supplier	Reference number
Acetonitrile	High Purity	Burdick & Jackson	CP 656
Formic acid	High Purity	Pro-analyst	K 34749064 524

Extraction procedure

- Thaw the plasma samples in a water bath at ~ 22 °C and vortex for 5 seconds.
- Aliquot plasma (250 µl) into Eppendorf tubes.
- Add 0.1 % formic acid in acetonitrile (200 µl) containing the internal standard (~ 2700 ng/ml proxyphylline) to the samples (not more than 4 samples at a time) and vortex for 30 seconds.
- Centrifuge the samples at 5750 G for 5 minutes.
- Transfer the supernatant to a 96-well plate and inject 3 µl onto the HPLC column using the chromatographic conditions given in Table 2.17.

Special precautions

Samples are cooled to ~ 5 °C on the autosampler while awaiting injection on instrument. Samples should be precipitated in groups of not more than 4 at a time.

For the first 1.6 minutes after injection, the mobile phase is diverted to waste before switching to the mass spectrometer.

Solutions

Formic acid solution (~ 0.1 %)

Dissolve formic acid (1 ml, 99 %) in water and make up to 1 000 ml with water.

Precipitation solution

Dissolve formic acid (0.1 ml, 99 %) in acetonitrile and make up to 100 ml with acetonitrile.

2.10.1.2 Spectra

Appendix C shows the mass spectrum of paracetamol (analyte), after collision, showing the protonated precursor ion at m/z 152.0, as well as the product ions. The mass spectrum of proxyphylline (internal standard), after collision, shows the protonated precursor ion at m/z 239.0, as well as the product ions.

2.10.1.3 Recording and Integration

Applied Biosystems Q-Trap LC-MS/MS detector at unit resolution in the multiple reaction monitoring (MRM) mode is used to monitor the transition of the m/z 152 and m/z 239 to the product ions m/z 110 and m/z 181 for paracetamol and the internal standard, respectively. Turbo ion spray (ESI) is used for ion production. The instrument is interfaced to a workstation running Analyst™ version 1.4.1 software.

Table 2.17: HPLC chromatographic conditions for paracetamol determination

Analytical column	Phenomenex® Luna Phenyl-Hexyl (5 µm) 150 × 2 mm column, fitted with a Phenomenex Security Guard™ guard cartridge system containing a C ₁₈ (5 µm), 4.0 x 2.0 mm I.D. guard cartridge.
Pump and flow rates	Agilent 1100 series pump delivering the mobile phase at a flow-rate of 200 µl/min at ambient temperature.
Mobile phase	Acetonitrile (40): 0.1 % formic acid solution (60) v/v
Injection	Agilent 1100 series autosampler injecting 3 µl onto the HPLC column
Detection	Applied Biosystems Q-Trap LC-MS/MS system using Turbo Ion Spray ionisation (ESI).
<u>Turbo Ion Spray Setting</u>	
Nebulizer gas (arbitrary value)	70
Turbo Spray (arbitrary value)	70
CUR (curtain gas) (arbitrary value)	20
CAD (collision gas) (arbitrary value)	3
TEM (source temperature) (°C)	400
IS (Ion Spray Voltage) (V)	5000

Table 2.17: Continued

<u>MS/MS Settings</u>	<u>Paracetamol</u>	<u>Proxyphylline</u>
Monoisotopic Mass	151.063	238.107
Protonated molecular mass (m/z)	152	239
Product ion molecular mass (m/z)	110	181
Dwell time (ms)	150	150
DP (declustering potential) (V)	40	40
EP (entrance potential)(V)	10	10
CEP (collision cell entrance potential) (V)	8	10
CE (collision energy) (eV)	21	21
CXP (collision cell exit potential) (V)	2	2
<u>Scan description</u>		
Scan type	MRM	
Polarity	Positive	
Pause time	5.0 minutes	
Retention times		
Paracetamol	2.04 – 2.12 minutes	Mean CV % = 0.5
Proxyphylline	1.92 – 2.01 minutes	Mean CV % = 0.5

2.10.1.4 Preparation of standards

Calibration standard

Calibration standards were prepared in plasma (anticoagulant lithium heparinate) by preparation of a stock solution in methanol and spiking a pool of normal blank plasma which was serially diluted with normal blank plasma to attain the desired concentrations (STD I - STD B). The preparation of the stock solution is given in Table 2.18. All volumetric operations were performed by weighing and the masses of plasma were converted to volumes when calculating concentrations. The calibration standards were prepared as shown in Table 2.19 and were aliquoted into polypropylene tubes and stored below -20°C .

Table 2.18: Preparation of stock solution for spiking STD I

Solvent used	SG solvent	Mass analyte	Mass solvent	Volume solvent	Volume spiked	Concentration analyte
Methanol	0.791	7.259 mg	11.009 g	13.918 ml	1 000 µl	521.56 µg/ml

Table 2.19: Preparation of calibration standards

Sample code and number	Source solution	A (g)	B (g)	C (g)	D (ng/ml)
STD I	Stock solution	21.340	61.340		13055
STD H	STD I	21.332	41.332	61.335	6527.8
STD G	STD H	21.321	41.321	61.327	3264.4
STD F	STD G	20.533	40.533	60.535	1632.3
STD E	STD F	20.565	40.566	60.569	816.18
STD D	STD E	20.591	40.592	60.592	408.08
STD C	STD D	20.483	40.484	60.483	204.03
STD B	STD C	21.075	41.075	61073	102.01

Note: Mass of biological fluid (g) is converted to volume (ml).

Density = 1.0269 kg/l for plasma.

A = Mass of empty container

B = Mass of container + normal blank plasma

C = Total mass of container + normal blank plasma + spiked plasma

D = Concentration of analyte

Quality control standards

Quality controls were prepared in plasma (anticoagulant lithium heparinate) using the same method that was used for the calibration standards. A new stock solution was prepared in methanol and used to spike a pool of normal blank plasma which was serially diluted with normal blank plasma to attain the desired concentrations (QC F - QC A) as shown in Tables 2.20 and 2.21. The quality control standards were aliquoted into polypropylene tubes and stored below -20 °C.

Table 2.20: Preparation of Stock solution for spiking QC F

Solvent used	SG Solvent	Mass analyte	Mass solvent	Volume solvent	Volume spiked	Concentration analyte
Methanol	0.791	8.286 mg	10.429 g	13.185 ml	2000 µl	628.46 µg/ml

Table 2.21: Preparation of Quality control standards

Sample code and number	Source solution	A (g)	B (g)	C (g)	D (ng/ml)
QC F	Stock solution	44.622	104.621		20801
QC E	QC F	45.261	85.261	125.262	10400
QC D	QC E	43.851	73.850	123.853	6500.5
QC C	QC D	45.123	99.121	107.269	852.28
QC B	QC C	45.073	83.572	105.082	305.50
QC A	QC B	45.483	85.490	105.480	101.79

Note: Mass of biological fluid (g) is converted to volume (ml).

Density = 1.0269 kg/l for plasma.

A = Mass of empty container

B = Mass of container + normal blank plasma

C = Total mass of container + normal blank plasma + spiked plasma

D = Concentration of analyte

Table 2.22: Parameters of the calibration curves

Run number	Calibration curve parameters			
	a	b	c	r ²
1	0.0239074268	0.482655635	-5.32907529	0.999234
2	0.0279099997	0.426802319	-5.10418710	0.999456
3	0.0240200029	0.478486601	-5.21524367	0.998891
4	0.0272786396	0.441028192	-5.10963662	0.999207
5	0.0244459468	0.467588808	-5.13830116	0.999321
6	0.0260239581	0.477414703	-5.33036929	0.998700
7	0.0278921096	0.425655444	-5.04356743	0.998920
8	0.0230491044	0.508546616	-5.37974076	0.999490
9	0.0196445734	0.552999416	-5.56296215	0.998876
10	0.0242550601	0.484776866	-5.29573040	0.999195
Mean	0.0248426821	0.474595460	-5.25088139	0.999129
CV %	10.2	8.1	-3.0	0.0
N	10	10	10	10

2.10.1.5 Quantification method

The results of paracetamol were processed using peak area ratios with Wagner calibration curves ($\ln(y) = a(\ln(x))^2 + b(\ln(x)) + c$) throughout this study.

Table 2.22 shows the calculated parameters of the calibration curves determined during the assay of the study samples as well as the coefficients of determination and correlation.

2.10.2 Determination of meloxicam in human plasma by LC-MS/MS

2.10.2.1 Quantification of study samples

Quantification of study samples was carried out using the assay method described in this report. During validation, this assay method provided an acceptable degree of accuracy and precision over the concentration range 10.17 - 1300 ng/ml based on peak area ratios with Wagner calibration curves ($\ln(y) = a(\ln(x))^2 + b(\ln(x)) + c$).

The reagents, grade, supplier and reference numbers of the reagent and chemicals used are given in Table 2.23.

Table 2.23: Reagents used for the determination of meloxicam

Reagent	Grade	Supplier	Reference number
Acetonitrile	High Purity	Burdick & Jackson	CP 656
Formic acid	High Purity	Pro-analyst	K 34749064 524

Extraction procedure

- Thaw plasma samples in a water bath at approximately 22 °C and vortex briefly to homogenise.
- Aliquot plasma (0.1 ml) into a 1.5 ml eppendorf tube and add 0.2 ml acetonitrile containing internal standard (piroxicam 300 ng/ml).
- Vortex samples for 30 seconds and centrifuge for 10 minutes at 8000 G.
- Transfer the supernatant layer to a 96 well plate.
- Inject 6 µl onto the HPLC column using the chromatographic conditions given in Table 2.24.

Special precautions

The samples are cooled to ~5 °C on the autosampler while awaiting injection on instrument. For the first two minutes after injection, the mobile phase is diverted to waste before switching to the mass spectrometer. Samples are precipitated in groups of not more than 4 at a time.

Solutions

Formic acid solution (~ 0.2 % v/v)

Add formic acid (2 ml, 98%) to water and make up 1 000 ml with water.

2.10.2.2 Spectra

Appendix C shows the mass spectrum of meloxicam (analyte) after collision, showing the protonated molecular ion at m/z 352.2, as well as the product ions. The mass spectrum of piroxicam (internal standard) after collision, showing the protonated molecular ion at m/z 332.0, as well as the product ions.

2.10.2.3 Recording and Integration

Applied Biosystems API 2000 mass spectrometer at unit resolution in the multiple reaction monitoring (MRM) mode is used to monitor the transition of the protonated precursor ions m/z 352 and m/z 332 to the product ions m/z 115 and m/z 95 for meloxicam and the internal standard, respectively. Electro Spray Ionisation (ESI) is used for ion production. The instrument is interfaced to a workstation running Analyst™ version 1.4.1 software.

Table 2.24: HPLC Chromatographic conditions for the determination of meloxicam

Analytical Column	Supelco Discovery® C ₁₈ , 5 µm, 150 × 2.1 mm column fitted with a Phenomenex precolumn (4 x 2 mm) packed with C18 packing. The column is kept at ~20 °C in an Agilent 1100 series column compartment.
Mobile Phase	Acetonitrile (50) : 0.2 % formic acid (50) v/v
Pump and Flow Rates	Agilent 1100 Series pump delivering the mobile phase at a flow-rate of 0.3 ml/min at ambient temperature.
Injection	An Agilent series 1100 series sampler, equipped with a 96-well tray, is used to inject 6 µl of each sample onto the column.
Detection	Applied Biosystems API 2000 LC-MS/MS system using Turbolon Spray ionization (ESI).
<u>Electro Spray Ionisation Setting</u>	
Nebulizer gas (Gas 1) (arbitrary unit)	70
CUR (curtain gas) (arbitrary unit)	10
CAD (collision gas) (arbitrary value)	8
TEM (source temperature) (°C)	400
IS (Ion Spray Voltage) (V)	4000
Turbo Spray gas (Gas 2)	70

Table 2.24: Continued

Table 2.24. Continued		
<u>MS/MS Settings</u>	<u>Meloxicam</u>	<u>Piroxicam</u>
Monoisotopic Mass	351	331
Protonated molecular mass (<i>m/z</i>)	352	332
Product ion molecular mass (<i>m/z</i>)	115	95
Dwell time (ms)	150	150
DP (declustering potential) (V)	11	6
FP (focusing potential)	360	370
EP (entrance potential)(V)	12	10.5
CEP (collision cell entrance potential)	26	22
(V)	27	35
CE (collision energy) (eV)	28	16
CXP (collision cell exit potential) (V)		
 <u>Scan description</u>		
Scan type	MRM	
Polarity	Positive	
Pause time	5.0 minutes	
 Retention times		
Meloxicam	3.26 – 3.36 minutes	Mean CV = 0.3 %
Piroxicam	2.37 – 2.43 minutes	Mean CV = 0.3 %

2.10.2.4 Preparation of standards

Calibration standards

Calibration standards were prepared in plasma (anticoagulant lithium heparinate) by preparation of a stock solution in dimethyl sulfoxide and spiking a pool of normal blank plasma which was serially diluted with normal blank plasma to attain the desired concentrations (STD I - STD B). The preparation of the stock solution is given in Table 2.25. All volumetric operations were performed by weighing and the masses of plasma were converted to volumes when calculating concentrations. The calibration standards

were prepared as shown in Table 2.26 and were aliquoted into polypropylene tubes and stored below -20 °C.

Table 2.25: Preparation of stock solution of meloxicam for spiking STD I

Solvent used	SG solvent	Mass analyte	Mass solvent	Volume solvent	Volume spiked	Concentration analyte
Dimethyl Sulfoxide	1.100	2.281 mg	6.173 g	5.612 ml	100 µl	406.46 µg/ml

Table 2.26: Preparation of Calibration standards

Sample code and number	Source solution	A (g)	B (g)	C (g)	D (ng/ml)
STD I	Stock solution	20.494	52.497		1300
STD H	STD I	21.297	37.298	53.309	650.2
STD G	STD H	20.661	36.662	52.670	325.2
STD F	STD G	21.265	37.268	53.280	162.6
STD E	STD F	20.638	36.639	52.641	81.32
STD D	STD E	20.507	36.505	52.517	40.68
STD C	STD D	21.276	37.274	53.277	20.34
STD B	STD C	20.612	36.612	52.619	10.17

Note: Mass of biological fluid (g) is converted to volume (ml)

Density = 1.0269 kg/l for plasma

A = Mass of empty container

B = Mass of container + normal blank plasma

C = Total mass of container + normal blank plasma + spiked plasma

D = Concentration of meloxicam

Quality control standards

Quality controls standards were prepared in plasma (anticoagulant lithium heparinate) using the same method that was used for the calibration standards. A new stock solution was prepared in dimethyl sulfoxide as shown in Tables 2.27 and 2.28 and used to spike a pool of normal blank plasma which was serially diluted with normal blank plasma to attain

the desired concentrations (QC G – QC A). The quality control standards were aliquoted into polypropylene tubes and stored below -20 °C.

Table 2.27: Preparation of stock solution of meloxicam for spiking QC F

Solvent used	SG solvent	Mass analyte	Mass solvent	Volume solvent	Volume spiked	Concentration analyte
Dimethyl Sulfoxide	1.100	3.808 mg	8.585 g	7.805 ml	200 µl	487.92 µg/ml

Table 2.28: Preparation of Quality control standards

Sample	Source	A	B	C	D
code & number	solution	(g)	(g)	(g)	(ng/ml)
QC G	Stock solution	20.695	68.700		2079
QC F	QC G	20.691	44.692	68.692	1039
QC E	QC F	20.608	44.608	68.614	519.7
QC D	QC E	21.189	52.190	69.200	184.1
QC C	QC D	20.490	52.493	68.495	61.38
QC B	QC C	21.310	53.312	69.309	20.46
QC A	QC B	20.594	44.597	68.594	10.23

Note: Mass of biological fluid (g) is converted to volume (ml).

Density = 1.0269 kg/l for plasma.

A = Mass of empty container

B = Mass of container + normal blank plasma

C = Total mass of container + normal blank plasma + spiked plasma

D = Concentration of meloxicam

2.10.2.5 Quantification method

The results of meloxicam were processed using peak area ratios with Wagner calibration curves ($\ln(y) = a(\ln(x))^2 + b(\ln(x)) + c$) throughout this study.

Table 2.29 shows the calculated parameters of the calibration curves determined during the assay of the study samples as well as the coefficients of determination and correlation.

Table 2.29: Parameters of the calibration curve

Run number	Calibration Curve Parameters			
	a	b	c	r ²
1	0.00254172541	0.993753217	-5.55738829	0.997239
2	-0.00229439913	1.06883112	-5.83325381	0.995464
3	-0.00501550115	1.07174504	-5.78308244	0.998861
4	0.00241158255	0.993477105	-5.56568119	0.998040
5	-0.00339974240	1.06447792	-5.69456392	0.998115
6	-0.0143640804	1.15604682	-5.84330937	0.999119
7	-0.00621730352	1.07558352	-5.71950709	0.997966
8	-0.00246064091	1.01864275	-5.48950069	0.998544
10	-0.00408588298	1.05532148	-5.63469856	0.998413
11	-0.00381485342	1.03198112	-5.49668019	0.999189
12	0.00146889267	0.998549794	-5.53797091	0.998511
13	-0.00306825322	1.04412578	-5.61807727	0.998968
14	-0.00628303999	1.06394133	-5.85220350	0.999274
Mean	-0.00342934588	1.04895977	-5.66353209	0.998285
CV %	-129.0	4.2	-2.4	0.1
N	13	13	13	13

2.10.3 Determination of codeine in human plasma by LC-MS/MS

2.10.3.1 Quantification of study samples

Quantification of study samples was carried out using the assay method described in this report. During validation, this assay method provided an acceptable degree of accuracy and precision over the concentration range 1.58 - 201 ng/ml based on peak area ratios with a Wagner calibration curves ($\ln(y) = a(\ln(x))^2 + b(\ln(x)) + c$).

1.33 mg codeine phosphate = 1 mg free codeine

The reagents and chemicals used, grade, supplier and reference numbers are given in Table 2.30 below.

Table 2.30: Reagents and chemicals used for the analysis of codeine

Reagent	Grade	Supplier	Reference number
Acetonitrile	High Purity	Burdick & Jackson	010
Ammonium acetate	98 %	Fluka	09690
Dichloromethane	High Purity	Burdick & Jackson	300
Ethyl acetate	High Purity	Burdick & Jackson	099
Formic acid	99 %	Fluka	524
Hexane	High Purity	Burdick & Jackson	216
Sodium hydroxide	Pro analysi	Fluka	1026605

Extraction procedure

- Thaw plasma samples in a water bath at ~ 22 °C and vortex briefly.
- Centrifuge for 3 minutes at 1300 G.
- Aliquot plasma (0.3 ml) into a 5 ml amber glass ampoule.
- Add internal standard solution (100 µl ~ 65 ng/ml naloxone in water).
- Add 200 µl of 0.1M Sodium hydroxide solution.
- Add hexane : dichloromethane : ethyl acetate (3 ml, 1 : 1 : 1 v/v).
- Vortex for 2 minutes and centrifuge at 1300 G for 3 minutes.
- Freeze the aqueous phase in an alcohol freezing bath (~ -30 °C) and decant the organic phase into a clean 5 ml amber glass ampoule.
- Evaporate to dryness under a gentle stream of nitrogen at ~ 40 °C.
- Add formic acid (2%, 200 µl) and vortex for 2 minutes.
- Transfer the extracts to 96-well plates.
- Inject 10 µl onto the HPLC column using the chromatographic conditions given in Table 2.31.

Special precautions

The samples are cooled to ~5 °C on the autosampler while awaiting injection on instrument.

Solutions

Ammonium acetate solution (0.01 M)

Dissolve ammonium acetate (0.77 g) in ~ 500 µl water and make up to a final volume of 1 000 ml with water.

Formic acid solution (2 %)

Add formic acid (20 ml) to ~ 500ml water and make up to a final volume of 1 000 ml with water.

Sodium hydroxide solution (0.1 M)

Add sodium hydroxide pellets (4.0 g) to ~500ml water and fill up to a final volume of 1 000 ml with water.

10.2.3.2 Spectra

Appendix C shows the mass spectrum of codeine (analyte) after collision, showing the protonated precursor ion at m/z 300.2, as well as the product ions. The following is a mass spectrum of naloxone (internal standard) after collision, showing the protonated precursor ion at m/z 328.4, as well as the product ions.

10.2.3.3 Recording and Integration

Applied Biosystems API 3000 mass spectrometer at unit resolution in the multiple reaction monitoring (MRM) mode is used to monitor the transition of the protonated precursor ions m/z 300 and m/z 328 to the product ions m/z 215 and m/z 310 for Codeine and the internal standard, respectively. Electro Spray Ionisation (ESI) is used for ion production. The instrument is interfaced to a workstation running Analyst™ version 1.4 software.

Table 2.31: HPLC chromatographic conditions for the determination of codeine

Analytical column	Supelco Discovery® C ₁₈ 150 × 2.1 mm, 5 µm analytical column, fitted with a Phenomenex SecurityGuard® system containing a C ₁₈ (4 × 3 mm) pre-column. The column is kept at ~ 30 °C with an Agilent 1100 series column compartment.	
Mobile phase	Acetonitrile: 0.01 M ammonium acetate (20 : 80 v/v), pH 3.5 with formic acid.	
Pump and flow rates	Agilent 1100 series pump delivering the mobile phase at a flow-rate of 300 µl/minute.	
Injection	An Agilent 1100 series autosampler, equipped with a 96-well tray, is used to inject 10 µl of each sample onto the column.	
Detection	Applied Biosystems Q-Trap LC-MS/MS system using Turbo Ion Spray ionisation (ESI).	
<u>Electron Spray Ionisation Setting</u>	<u>Value</u>	
Nebulizer gas (Gas 1) (arbitrary unit)	10	
CUR (curtain gas) (arbitrary unit)	6	
CAD (collision gas) (arbitrary unit)	12	
TEM (source temperature) (°C)	500	
IS (Ion Spray Voltage) (V)	3000	
<u>MS/MS Settings</u>	<u>Codeine</u>	<u>Naloxone</u>
Monoisotopic mass	299.2	327.2
Protonated molecular mass (m/z)	300	328
Product ion molecular mass (m/z)	215	310
Dwell time (ms)	75	75
DP (declustering potential) (V)	46	41
FP (focusing potential) (V)	200	200
EP (entrance potential) (V)	10	10

Table 2.31: Continued

<u>MS/MS Settings</u>	<u>Codeine</u>	<u>Naloxone</u>
CE (collision energy) (eV)	35	27
CXP (collision cell exit potential) (V)	12	18
<u>Scan description</u>		
Scan type	MRM	
Polarity	Positive	
Pause time	5 minutes	
Retention times	1.90 – 2.00 minutes	Mean CV = 0.2 %
Codeine	1.80 – 1.90 minutes	Mean CV = 1.2 %
Naloxone		

2.10.3.4 Preparation of standards

Calibration standards

Calibration standards were prepared in plasma (anticoagulant lithium heparinate) by preparation of a stock solution in methanol and spiking a pool of normal blank plasma which was serially diluted with normal blank plasma to attain the desired concentrations (STD I - STD B). All volumetric operations were performed by weighing and the masses of plasma were converted to volumes when calculating concentrations. The calibration standards were aliquoted into polypropylene tubes and stored below -20 °C.

Mass of reference standard weighed: codeine 1.488 mg

Table 2.32: Preparation of Stock solution for spiking STD I

Solvent used	SG solvent	Mass analyte	Mass solvent	Volume solvent	Volume spiked	Concentration analyte
Methanol	0.791	1.121 mg	11.294 g	14.278 ml	250 µl	78.51 µg/ml

Table 2.33: Preparation of Calibration standards

Sample code and number	Source solution	A (g)	B (g)	C (g)	D (ng/ml)
STD I	Stock solution	45.099	145.080		201
STD H	STD I	45.529	95.577	145.135	100
STD G	STD H	43.894	93.823	143.719	50.0
STD F	STD G	45.353	95.368	145.581	25.1
STD E	STD F	45.989	95.857	146.211	12.6
STD D	STD E	45.169	95.078	146.302	6.38
STD C	STD D	45.081	95.085	144.870	3.18
STD B	STD C	45.557	95.551	145.077	1.58

Note: Mass of biological fluid (g) is converted to volume (ml)

Density = 1.0269 kg/l for plasma

A = Mass of empty container

B = Mass of container + normal blank plasma

C = Total mass of container + normal blank plasma + spiked plasma

D = Concentration of analyte

Quality control standards

Quality controls were prepared in plasma (anticoagulant lithium heparinate) using the same method that was used for the calibration standards. A new stock solution was prepared as per Tables 2.34 and 2.35 in methanol and used to spike a pool of normal blank plasma which was serially diluted with normal blank plasma to attain the desired concentrations (QC F - QC A). The quality control standards were aliquoted into polypropylene tubes and stored below -20 °C.

Mass of reference standard weighed: codeine 1.752 mg

Table 2.34: Preparation of Stock Solution for Spiking QC F

Solvent used	SG Solvent	Mass analyte	Mass solvent	Volume solvent	Volume spiked	Concentration analyte
Methanol	0.791	1.320 mg	10.400g	13.148 ml	500 µl	100.40 µg/ml

Table 2.35: Preparation of Quality control standards

Sample code and number	Source Solution	A (g)	B (g)	C (g)	D (ng/ml)
QC F	Stock solution	44.700	204.709		321
QC E	QC F	43.860	123.863	203.853	161
QC D	QC E	45.510	125.520	205.535	80.3
QC C	QC D	44.542	189.536	205.232	7.84
QC B	QC C	45.046	125.016	205.016	3.92
QC A	QC B	45.506	125.505	178.499	1.56

Note: Mass of biological fluid (g) is converted to volume (ml).

Density = 1.0269 kg/l for plasma.

A = Mass of empty container

B = Mass of container + normal blank plasma

C = Total mass of container + normal blank plasma + spiked plasma

D = Concentration of analyte

2.10.3.5 Quantification method

The results of codeine were processed using peak area ratios with a Wagner calibration curve ($\ln(y) = a(\ln(x))^2 + b(\ln(x)) + c$) throughout this study.

Tabulated below in Table 2.38 are the calculated parameters of the calibration curves determined during the assay of the study samples as well as the coefficients of determination and correlation.

Table 2.36: Parameters of the calibration curves

Run number	Calibration curve parameters			
	a	b	c	r ²
1	0.0153475514	0.936254547	-4.60390170	0.997308
2	0.00232231013	1.00688081	-4.63214073	0.996434
3	0.00707870487	0.963917326	-4.66226253	0.997499
4	0.000919889445	0.989084248	-4.60815610	0.998134
6	-0.00129722167	0.986157281	-4.43183494	0.999305
5	0.0138228159	0.917507720	-4.54452884	0.999081
7	-0.00205838127	1.02208430	-4.58061752	0.998854
8	0.00899822753	0.948985489	-4.61017983	0.998746
10	0.00360109791	0.976351462	-4.57368945	0.998614
11	0.0152317599	0.903126999	-4.49140438	0.999216
12	0.0144574724	0.922034554	-4.56436624	0.998908
13	-0.00407263579	1.00529530	-4.54049076	0.996829
14	0.00317456974	0.964530075	-4.45074352	0.999428
15	0.00371063681	0.965008329	-4.37686119	0.999348
Mean	0.00580262838	0.964801317	-4.54794127	0.998407
CV %	116	3.7	-1.8	0.1
N	14	14	14	14

2.10.4 Calculation of results

Analysed samples which do not pass the chromatographic acceptance criteria are identified as poor chromatograms and are coded (Watson™ LIMS code “C”) in the regression menu, toggled as “Not Reportable” and tagged for re-assay. Such samples are repeated in single fold and the repeated value is reported as the final value if it meets the acceptance criteria. Analysed samples deemed unacceptable due to technical errors such as instrument failure, excessive internal standard aberrations or accidental sample loss or destruction are identified as lost in process and are coded (Watson™ LIMS code “B”) in the regression menu, toggled as “Not Reportable” and tagged for re-assay. Such samples are performed

in single fold and the repeated value is reported as the final value if it meets the acceptance criteria.

When the calculated concentration of a study sample exceeds the concentration of the highest nominal calibration standard, the sample is automatically flagged as being above limit of quantification (ALQ) and is coded (Watson™ LIMS code “F”) in the regression menu, toggled as “Not Reportable” and tagged for re-assay. The sample is diluted with drug-free matrix to bring the concentration within the standard curve range and the analysis is repeated in single fold. The dilution factor is then used to calculate and report the final concentration.

If, in any of the repeat assays described above, the requirements are not met due to repeated poor chromatography or technical error, the value is reported as not reportable (Watson™ LIMS code “NR3”) and “NR3” is reported in the table of individual study sample concentrations.

CHAPTER 3

SYNERGISTIC NOCICEPTIVE ACTIVITY IN RATS

INTRODUCTION

The antinociceptive activity of meloxicam, codeine phosphate and paracetamol in rats was investigated at the Brain Function Research Unit (BFRU) of the University of the Witwatersrand. This study was published by Norris (2005:United States Patent 2005/0090517) for a United States patent application on behalf of Adcock Ingram. Skill advancement in the BFRU allowed the combination to be administered orally rather than intraperitoneally. This would be the preferred route of administration for a commercial product for use in humans.

The assay consisted of a battery test developed over 20 years. It has shown to be useful in estimating the potencies of cyclo-oxygenase (COX)-inhibitors marketed as nonsteroidal anti-inflammatory drugs (NSAIDs) (Gregor *et al.*, 1992:412-416). The scientific foundation of the battery has recently been validated independently at a distinguished pain-research laboratory in Australia (Grace *et al.*, 2001:117-125). The battery includes a modified tail flick test of sensitivity to noxious ischemia, by occluding the blood supply to the tail and a measurement of the hyperalgesia which develops during reperfusion to tissue following transient ischaemia (Gelgor *et al.*, 1986:251-257).

A hyperalgesic state can be defined as the state of exaggerated response to painful stimuli. It is a behavioral state in which the threshold to potentially painful events is reduced and reactions to supra-threshold painful events are exaggerated. Algesia describes a normal physiological response to pain. The battery distinguishes analgesia as that produced for example by morphine (Sher *et al.*, 1992:241-248), from the antihyperalgesia produced by COX-inhibitors (Mitchell, 1999:463-469). It also identifies primary antinociceptive activity, rather than antinociceptive activity that might appear secondarily following resolution of inflammation.

Because any test of antinociception in animals may produce false positive results if the antinociceptive agent under investigation compromises the animal's motor function (Cartmell *et al.*, 1991:140-159), it usually is necessary to check the activity of any agents under test on motor function. However, previous work carried out by the BFRU has established that at the doses under investigation, none of the three agents have any effect on motor function, thus the checks for motor function were waived.

No scientific evidence upon which to constitute an appropriate ratio of meloxicam, codeine phosphate and paracetamol was found. It was decided to employ 1 meloxicam: 4 codeine phosphate: 266 paracetamol. The ratio was derived from the maximum daily doses of the three agents routinely employed clinically in South Africa namely 15 mg meloxicam, 60 mg codeine phosphate and 4 000 mg paracetamol. If antinociceptive activity could be demonstrated at these doses, it might be possible to retreat the high doses in follow up investigations.

3.1 Ethical considerations

The experimental procedures were approved by the Animal Ethics Committee of the University of the Witwatersrand (Certificate No. 2002/50/3) and complied with the recommendations of the Committee for Research and Ethical issues of the International Association for the study of pain (Zimmerman, 1983:109-110).

3.2 Method

3.2.1 Animals

Both genders of Sprague-Dawley rats (*Rattus norvegicus*) weighing 250 - 450 g were used for nociceptive tests. The rats were housed individually at an ambient temperature of 22 °C $\pm$ 1 °C on a 12-hour light - 12-hour dark cycle and were allowed free access to standard rat feed and tap water. Groups of ten rats were used for each test. Rats were returned to stock in good condition and health after the test.

3.2.2 Test for nociception

3.2.2.1 Nociception during noxious ischaemia

Ischaemia was induced by applying an inflatable tourniquet to the base of the restrained rat's tail, as detailed by Gelgor *et al.* (1992:412-416). The moment the rat exhibited an escape response, the tourniquet was deflated. The time between application of a tourniquet and the escape response, is the escape latency which is a measure of sensitivity of the noxious stimulus induced by ischaemia. To prevent tissue damage, the tourniquet was removed if the rat had not responded within 30 minutes.

3.2.2.2 Nociception during noxious thermal stimulation

The response of the rat to a noxious thermal stimulus was measured using a tail flick test as detailed by Gelgor *et al.* (1992:412-416). The rat's tail was submerged in a water bath controlled at 49 °C and the time of the first coordinated motor response of the tail measured, with a safety cut off at 30 seconds. Tail skin temperature was maintained at 29 °C by placing all but the proximal 20 mm of the rat's tail in a temperature controlled bath, before and between measurements, to obviate the potential confounding effects of changes in tail flick latency (Tjolsen *et al.*, 1989:259).

3.2.2.3 Hyperalgesia

The hyperalgesia that occurs during reperfusion of the tail following transient ischemia, that is, following the release of the tourniquet mentioned above, was measured by comparing the tail flick latency during reperfusion with that measured in the same animal before application of the tourniquet. Hyperalgesia is manifested as reduced tail flick latency during reperfusion and lasts about one hour (Gelgor *et al.*, 1992:412-416). Control experiments were performed by placing a sham tourniquet on the tail for 20 minutes (mean escape latency measured in previous experiments in the laboratory) and administering the highest dose of each agent or combination thereof.

3.2.3 Procedure

The rats were habituated to restrainers on 3 hours per day on three consecutive days before any measurements were made. On the second of these days, the rats were given

an oral dose of water, to allow them to experience the feeding tubes in advance. On experimental days, the animals were placed in restrainers for at least 30 minutes before any testing. At least 48 hours were allowed between successive measurements on individual animals. Experiments were carried out during the day (when rats are normally quiescent), at an ambient temperature of 24 °C.

The tail flick latency (mean of three measurements, 1 minute apart) was measured and then the agent under investigation was administered. Thirty minutes later, the tourniquet (or sham tourniquet) was applied and the escape latency measured. Immediately after release of the tourniquet, the tail flick latency was again measured and the measurement was repeated after 30 and 60 minutes of reperfusion. Following inspection of the data at all three periods of reperfusion, the investigators elected to base their investigation on antihyperalgesic activity on data recorded immediately after release of the tourniquet.

3.2.3.1 Administration of test substances

Meloxicam was obtained from Neolux Limited, paracetamol and codeine phosphate from Fine Chemicals. Meloxicam was dissolved in sunflower oil, codeine phosphate in water and paracetamol suspended in sunflower oil. All three agents were administered in boluses of 0.2 ml and the paracetamol suspension was flushed with water. In control experiments, the appropriate vehicle (water, sunflower or both) was administered.

Each agent was administered separately, at three doses, to determine the antinociceptive activity, if any, of each independently. Pairs of agents were then administered at three doses for each pair. Finally, the combination of the three agents was administered at three doses. In all cases, the ratio of the agents was fixed at 1 meloxicam: 4 codeine phosphate: 266 paracetamol. The targeted maximum dose of meloxicam was 5 mg/ kg. Rats are less sensitive to all COX-inhibitors than humans, for which the corresponding dose of paracetamol would have been 1333 mg/kg. The actual doses were based on unpublished data on the antipyretic activity of meloxicam administered orally in rats. It was not possible to make the suspension of paracetamol to administer at such a high dose, so the targeted maximum dose of 5 mg/kg meloxicam and 20 mg/kg codeine phosphate were employed only when the combination with paracetamol was required.

Each group of ten rats received all doses and the appropriate control administration, for just one agent or combination of agents which exposed each rat to five oral administrations after the pilot administration. The number of administrations in each rat was limited, not as a result of fear to potential adverse events or toxicity but to maintain the experimental animals in the same mass range throughout the study.

3.3 Results

3.3.1 Analgesic (opioid-like) activity

Table 3.1 shows the escape latencies (the measure of sensitivity of noxious ischaemia stimulus) following administration of the highest dose tested for each agent, or combination of agents, together with the escape latency for the same group of rats following a demonstration of the appropriate vehicle. Because escape latencies vary between groups of rats, the efficacy of each active agent must be judged by comparing the two escape latencies within the same group of rats. Although escape latencies appeared to increase following administration of several of the agents and combinations, the only administration which produced a statistically significant increase in escape latency, that is statistically significant analgesia to noxious ischaemia, was a combination of meloxicam 5 mg/kg and codeine phosphate 20 mg/kg.

Table 3.1: Escape latency (median 95 % confidence interval, n=10)

Agent	Dose (mg/kg)	Escape latency (minutes)	
		Agent	Vehicle
Meloxicam	5	25.3 (15.7 – 30.0)	19.3 (11.2 – 30.0)
Codeine phosphate	20	20.9 (11.9 – 30.0)	26.7 (11.7 – 30.0)
Paracetamol	266	18.2 (9.2 – 30.0)	17.2 (11.3 – 30.0)
Meloxicam	5	25.1 (13.6 – 27.1)*	13.3 (8.8 – 21.2)
Codeine phosphate	20		
Meloxicam	1	23.6 (10.9 – 30.0)	15.0 (8.4 – 30.0)
Paracetamol	266		

Table 3.1: Continued

Agent	Dose (mg/kg)	Escape latency (minutes)	
		Agent	Vehicle
Paracetamol	266	24.9 (9.1 – 30.0)	16.7 (9.9 – 30.0)
Codeine phosphate	4		
Meloxicam	1	17.9 (10.8 – 27.0)	16.4 (8.7 – 30.0)
Codeine phosphate	4		
Paracetamol	266		

* Significant increase, $p = 0,004$ (Friedman Test)

Figure 3.1 shows the relationship between the escape latency and dose for the meloxicam/codeine phosphate combination and demonstrates that, at the highest dose escape latency was increased by about 90 %, which is evident of substantial analgesic activity. The graph was plotted following oral administration of meloxicam: codeine phosphate mixture in the ratio 1:4. Data is plotted against the codeine phosphate dose ED_{50} of 2.5 mg/kg meloxicam and 10 mg/kg codeine phosphate (fit standard error = 12 %, log-linear regression analysis).

If the combination of meloxicam and codeine phosphate produces analgesia, it would be expected that the triple combination formed by adding paracetamol would produce analgesia too. However, this could not be tested, because the dose of paracetamol (1333 mg/kg) corresponding to the 20 mg/kg dose of codeine phosphate was physically too large to administer. At the highest dose for the combination that could be tested, in which the codeine phosphate contribution was 4 mg/kg, there was no significant analgesia.

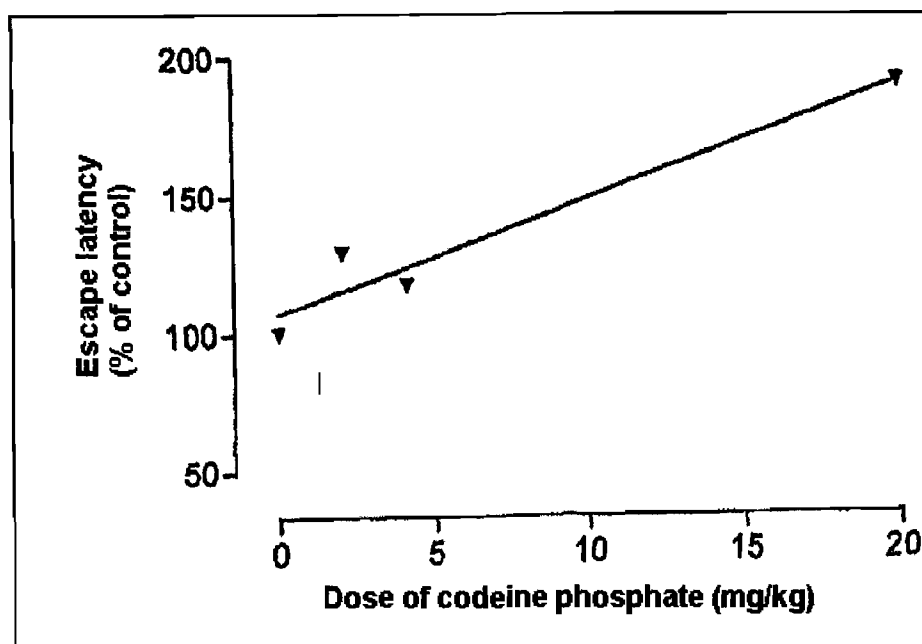


Figure 3.1: A graphical representation of escape latency (median, n=10), in the same group of rats

3.3.2 Antihyperalgesic (COX-inhibitor-like) activity

Antihyperalgesia is measured by the extent to which an agent reverses the hyperalgesia induced by the conditioning event, which, in this case was transient ischaemia. An antihyperalgesic index of zero means complete lack of antihyperalgesic activity, an index of 100 % means full reversal of the hyperalgesia, and an index of more than 100 % implies some analgesic activity in addition to the antihyperalgesic activity.

Figure 3.2 shows the antihyperalgesic index, at three doses of each of the agents administered separately. The antihyperalgesic index was calculated according to the formula of Sher *et al.* (1992:241) during reperfusion of the rat tail following transient ischaemia, after oral administration of the three agents. At the doses tested, which were limited to 266 mg/kg, paracetamol did not exhibit any antihyperalgesic activity. Even though their doses were much lower, both meloxicam and codeine phosphate did exhibit dose dependent antihyperalgesia activity, even when administered separately.

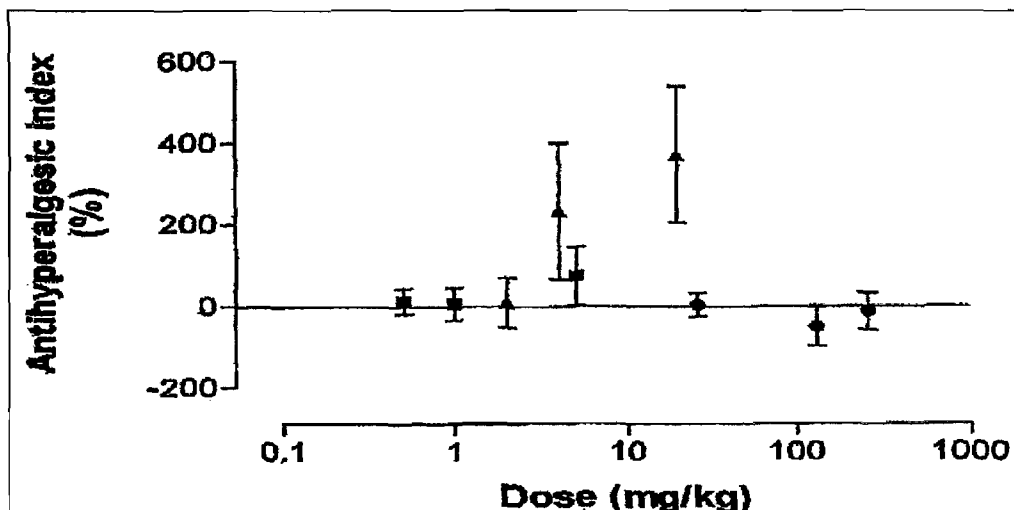


Figure 3.2: A graphical representation of an antihyperalgesic index (n=9 or 10, different animals for each agent), meloxicam (squares), codeine phosphate (triangles) and paracetamol (circles)

Inspection of Figure 3.2 shows regression analysis and confirms that at a dose of 0.22 mg/kg, meloxicam on its own showed no antihyperalgesic activity. Similarly, neither did codeine phosphate at 0.88 mg/kg nor paracetamol at 59 mg/kg. Thus in combination, the three components showed antihyperalgesic activity at doses at which the components were individually inactive, which is an indication of their synergistic activity when in combination. The antihyperalgesic activity of the individual components was also tested in pairs, rather than as a triple combination. At the doses tested, paracetamol and codeine phosphate together had no significant antihyperalgesic activity.

Figure 3.3 shows the antihyperalgesic index exhibited when the triple combination of meloxicam, codeine phosphate and paracetamol was administered in the ratio 1:4:266 respectively. Data is plotted against the meloxicam dose. Dose dependent antihyperalgesia was evident, even though the doses of the individual agents in the combination were much lower than those required to produce antihyperalgesia separately. An ED_{50} value (dose at which reperfusion hyperalgesia is reduced by 50 %) was calculated from regression analysis applied to the data, and is shown, together with the ED_{50} values for the individual agents, in Table 3.2 (ED_{50} for the antihyperalgesic efficacy from the least squares regression lines fitted to the dose-response curves). The ED_{50} was 0.22 mg/kg meloxicam

+ 0.88 mg/kg codeine phosphate + 59 mg/kg paracetamol.

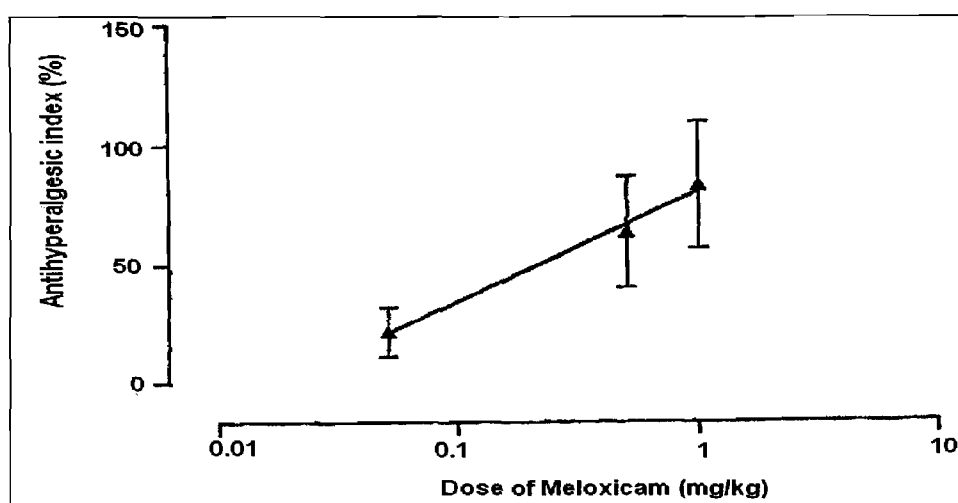


Figure 3.3: A graphical representation of an antihyperalgesic index (n= 9 or 10) during reperfusion of the rat tail following transient ischaemia, (fit standard error= 4 %, log-linear regression analysis)

Table 3.2: ED₅₀ for the antihyperalgesic efficacy (n=9 or 10 for each test)

Agent	ED ₅₀ (mg/kg)
Meloxicam	0.22
Codeine phosphate	0.88
Paracetamol	59
Meloxicam	2.6
Codeine phosphate	2.4
Paracetamol	> 266

Meloxicam and codeine phosphate did have significant antihyperalgesic activity, with an ED₅₀ of 1.7 mg/kg meloxicam and 6.8 mg/kg codeine phosphate, which is about ten times higher than the ED₅₀ doses in the triple combination. Meloxicam and paracetamol also had antihyperalgesic activity, and with an ED₅₀ not significantly different from that of the triple combination. But the investigators did not have confidence in the ED₅₀ value calculated for the meloxicam and paracetamol pair because of high inter-individual variability of the rats in

that

particular

cohort.

3.3.3 Adverse events

Formal tests for adverse events were not part of the study design. However, the animals remained alert, active and in apparent good health throughout the study. Also, all animals continued to gain weight throughout the study; loss of weight is an early sign of pathology in rats. The animals were housed in the Central Animal Service of the University under veterinary care and no adverse events were recorded on the veterinary bed letters. As far as the active ingredients tested were concerned, no interactions between the actives of an adverse nature were observed.

3.4 Conclusions

From the findings above it was demonstrated that a triple combination of meloxicam, codeine phosphate and paracetamol, administered orally in a single dose to rats, produced significant antihyperalgesic activity, which is the ability to relieve pain of the type which occurs in indications for which the cyclo-oxygenase inhibitors are functional. The dose of meloxicam required when in combination with codeine phosphate and paracetamol is about one tenth of the dose of meloxicam required if it is administered as a single agent. This means that combining meloxicam with codeine phosphate and paracetamol enhances the antihyperalgesic efficacy of meloxicam by about ten-fold. This enhancement results from synergies between the three components, because the ED₅₀ of the triple combination is composed of doses at which each of the three components is inactive on its own. Thus the triple combination of meloxicam, codeine phosphate and paracetamol is a candidate for a synergistic antihyperalgesic product.

Although no formal assessments of adverse events associated with the administration of the triple combination was made, each rat in the relevant cohort received the triple combination on four occasions in various doses, which included twice receiving a dose five times higher than the ED₅₀. The investigators did not observe a change in the weight gain, demeanour, activity nor vital signs of the rats, and they were in excellent condition at the end of the study. There was no reason to expect synergy of adverse events in the triple

combination.

The above also demonstrates that a combination of meloxicam and codeine phosphate produces significant analgesic activity that is ability to relieve pain of the type which occurs in indication for which the opioids are active. The ED₅₀ dose of meloxicam and codeine phosphate necessary to produce significant analgesic activity was about ten times higher than their contributions in the ED₅₀ dose of the triple combination for antihyperalgesic activity. Physical constraints related to the insolubility of paracetamol prevented the investigation of whether addition of paracetamol, in the appropriate ratio, to the meloxicam and codeine phosphate combination would enhance the analgesic activity. However, it is believed to be highly unlikely that the paracetamol would reduce analgesic activity, thus, by extrapolation, the triple combination of meloxicam, codeine phosphate and paracetamol will produce significant opioid-like analgesic activity, but only at doses much higher than the doses necessary for antihyperalgesic activity.

As the triple combination provides both antihyperalgesic (cyclo-oxygenase inhibitor-like) and analgesic (opioid-like) activity, it is a candidate for a medication indicated for a wide range of types of pain, both conditioned pain (e.g. pain and inflammation, dysmenorrhoea) and unconditioned pain (e.g. non-inflammatory myalgia). The combination of meloxicam and codeine phosphate, without any paracetamol, produced significant opioid-like analgesia, but needed a high dose of codeine phosphate.

The synergistic effect of the active ingredients is believed to can provide a pharmaceutical product which delivers high antihyperalgesic effects while each active may be at a sub-therapeutic dose. This, it is believed, will provide a product with a low side effect profile. Furthermore due to the low doses of the actives the pharmaceutical product cost for treating hyperalgesic states could be reduced.

CHAPTER 4

PRODUCT DEVELOPMENT AND STABILITY

INTRODUCTION

The formulation of this fixed dose combination product was developed at Adcock Ingram's Research and Development site which is a current Good Manufacturing Practice (cGMP) approved facility. The fixed dose combination capsule formulation was based on the synergistic analgesia and antihyperalgesic activity observed in Chapter 3 when the three actives were used for combination in animal studies. The strength of the active ingredients in the capsule to be developed is 250 mg paracetamol, 10 mg codeine phosphate and 2.5 mg meloxicam. This combination will be referred to as the Triple Fixed Dose Combination (FDC) capsules throughout the text. Three variations of this product were required for the proof of concept clinical trial: the Triple FDC, Double FDC (paracetamol 250 mg and codeine phosphate 10 mg) and placebo. The proof of concept study required blinding thus, the final product for all variants needed to be similar in appearance, i.e. similar capsule shell or coated tablets.

4.1 Pharmacological properties

4.1.1 Meloxicam

Meloxicam, an oxicam derivative, is a NSAID. It is used in the management of rheumatoid arthritis, for the short-term symptomatic treatment of acute exacerbations of osteoarthritis and for the symptomatic treatment of ankylosing spondylitis.

Meloxicam is well absorbed after oral doses and is 99 % bound to plasma proteins. Meloxicam has an elimination half-life of approximately 20 hours. It is extensively metabolised by oxidation and excreted in similar amounts in the urine and in the faeces; less than 3 % of the dose is excreted unchanged. The volume of distribution is increased in renal failure. Meloxicam should be avoided in patients with severe hepatic impairment, bleeding disorders and in patients with renal failure unless receiving dialysis.

The most common side-effect of NSAIDs is generally gastrointestinal disturbances, such as gastrointestinal discomfort, nausea and diarrhea. These are usually mild and reversible but in some patients, peptic ulceration and severe gastrointestinal bleeding may occur (Sweetman, 2007:68).

The oral pharmacokinetic parameters of meloxicam in healthy mexican subjects compared with historic controls reported in other populations, showed a reduced clearance and volume of distribution when compared with german subjects, whereas no differences between mexican and chinese subjects were observed. These results suggest that there are inter-ethnic differences in the pharmacokinetics of meloxicam. Carrasco-Portugal (2005:307-313) suggested that blind extrapolation of dosage regimens from one population to another should be avoided due to the risk of unexpected incidences of adverse effects.

4.1.2 Paracetamol

Paracetamol is a para-aminophenol derivative with analgesic and antipyretic activities. It is given by mouth or as a rectal suppository for mild to moderate fever. Paracetamol is the analgesic or antipyretic of choice in patients in whom salicylates or other NSAIDs are contra-indicated.

Paracetamol is readily absorbed from the gastrointestinal tract with peak plasma concentrations occurring 10 to 60 minutes after oral doses. It is distributed into most body tissues. Plasma protein binding is negligible at usual therapeutic concentrations. The elimination half-life of paracetamol is 1 to 3 hours. Paracetamol is metabolised predominantly in the liver and excreted in the urine mainly as the glucuronide and sulfate conjugate. Less than 5 % is excreted unchanged as paracetamol. It should be given with care to patients with impaired kidney or liver function or those with alcohol dependence. The side effects of paracetamol are usually rare and mild, although haematological reactions have been reported (Sweetman, 2007:92-95).

4.1.3 Codeine phosphate

Codeine, a phenanthrene derivative, is an opioid analgesic obtained from opium or made by methylating morphine. It is much less potent as an analgesic than morphine and has relatively mild sedative effects. Codeine or its salts, especially the phosphate, are given by mouth in the form of linctuses for the relief of cough and as tablets for the relief of mild to moderate pain, often in association with a non-opioid analgesic such as aspirin, ibuprofen or paracetamol. For the relief of pain, codeine phosphate may be given in doses of 30 to 60 mg every 4 hours to a maximum dose of 240 mg daily.

In therapeutic doses, codeine is much less liable than morphine to produce adverse effects, although constipation may be troublesome with long term use. Codeine like morphine, has a dose related histamine-releasing effect. After large doses of codeine, excitement and convulsions may occur (Sweetman, 2007:32-33). Jung (2004:1037) reported on the conversion of codeine to the more active drug morphine. However, this conversion was not equal in all patients or ethnic groups.

4.2 Physicochemical properties of the active ingredients

The physicochemical properties of meloxicam, paracetamol and codeine phosphate are listed in Table 4.1.

4.3 Inactive ingredients

The inactive ingredients used in the products are commonly used in commercially available pharmaceutical preparations containing paracetamol and codeine phosphate and those with only meloxicam. These ingredients are listed in Table 4.2 together with their functions and safety profiles. The inactive ingredients used in the formulation are generally regarded as safe and devoid of toxic activity when used within the recommended doses. It is unlikely that the excipients used in the formulation will contribute negatively to the safety profile of the product. Although sodium lauryl sulphate has the potential to exhibit toxicity, the concentration used in the formulation has no potential to produce untoward effects.

Table 4.1: Physicochemical properties of the active ingredients (BP, 2007)

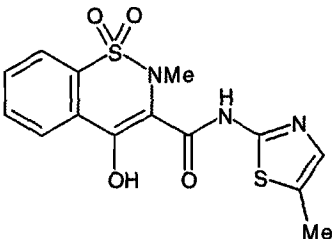
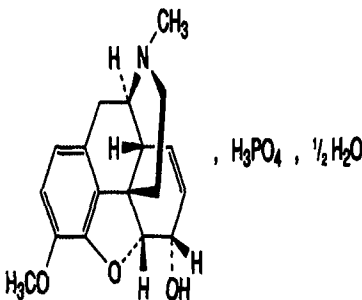
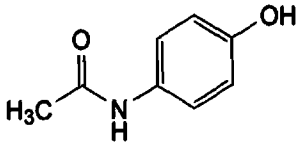
Active ingredient	Chemical Structure, molecular formula and molecular mass	Characteristics	Solubility
Meloxicam	 Molecular formula: $C_{14}H_{13}N_3O_4S_2$ Molecular mass: 351.4	A pale yellow powder	Practically insoluble in water. Slightly soluble in acetone. Soluble in dimethylformamide. Very slightly soluble in ethanol (96 %) and in methanol.
Codeine phosphate	 Molecular formula: $C_{18}H_{21}NO_3, H_3PO_4, \frac{1}{2}H_2O$ Molecular mass: 406.4	White or almost white, crystalline powder or small, colourless crystals	Freely soluble in water. Slightly soluble or very slightly soluble in alcohol (96 %).
Paracetamol	 Molecular formula: $C_8H_9NO_2$ Molecular mass: 151.2	White, crystalline powder	Sparingly soluble in water. Freely soluble in alcohol. Very slightly soluble in methylene chloride.

Table 4.2: Properties of inactive ingredients used in the manufacturing of the FDC capsules (Kibbe, 2000)

Ingredient	Functional Category	Safety
Povidone (Polyvinylpyrrolidone K 30)	Binder and dissolution aid	When consumed orally, povidone may be regarded as essentially nontoxic since it is not absorbed from the gastrointestinal tract or mucous membranes.
Pregelatinised starch	Disintegrant and binder	Generally regarded as a nontoxic and nonirritant excipient. However, oral consumption of massive amounts may be harmful.
Sodium lauryl sulphate (SLS)	Surfactant	A moderately toxic material with acute toxic effects including irritation to the skin, eyes, mucous membrane, upper respiratory tract and stomach.
Magnesium stearate	Lubricant	Generally regarded as nontoxic and nonirritant excipient following oral administration. Oral consumption of large quantities may result in some laxative effect or mucosal irritation.
Purified water	Solvent	Ingestion of excessive quantities can lead to water intoxication with disturbances of electrolyte balance.
Lactose	Capsule diluent	Adverse reactions of lactose are largely attributed to lactose intolerance. This results in lactose being undigested and may lead to clinical symptoms including abdominal cramps, diarrhea, distention and flatulence.
Microcrystalline cellulose	Diluent and disintegrant	Microcrystalline cellulose is not absorbed systemically following oral administration and thus has little toxic potential.
Crospovidone	Disintegrant	Generally regarded as nontoxic and nonirritant. Short-term animal toxicity studies have shown no adverse effects.
Colloidal silicon dioxide (Aerosil 200)	Glidant	Generally regarded as essentially nontoxic and nonirritant.

4.4 Preformulation

4.4.1 Incompatibilities and possible interactions

There are no expected interactions between the inactive ingredients and the active ingredients or between the active ingredients themselves. Numerous products currently registered within Adcock Ingram containing the combination of paracetamol and codeine phosphate, with the proposed excipients have been subjected to stability studies that resulted in no apparent incompatibilities.

The compatibility of codeine phosphate with the inactive and active ingredients used in the formulation was assessed using the DSC method described in Chapter 2.

Codeine phosphate was mixed in a 1:1 ratio with either the active or inactive ingredients and the compatibility of the mixture was assessed. The DSC thermograms of some of the mixtures are shown in Figure 4.1.

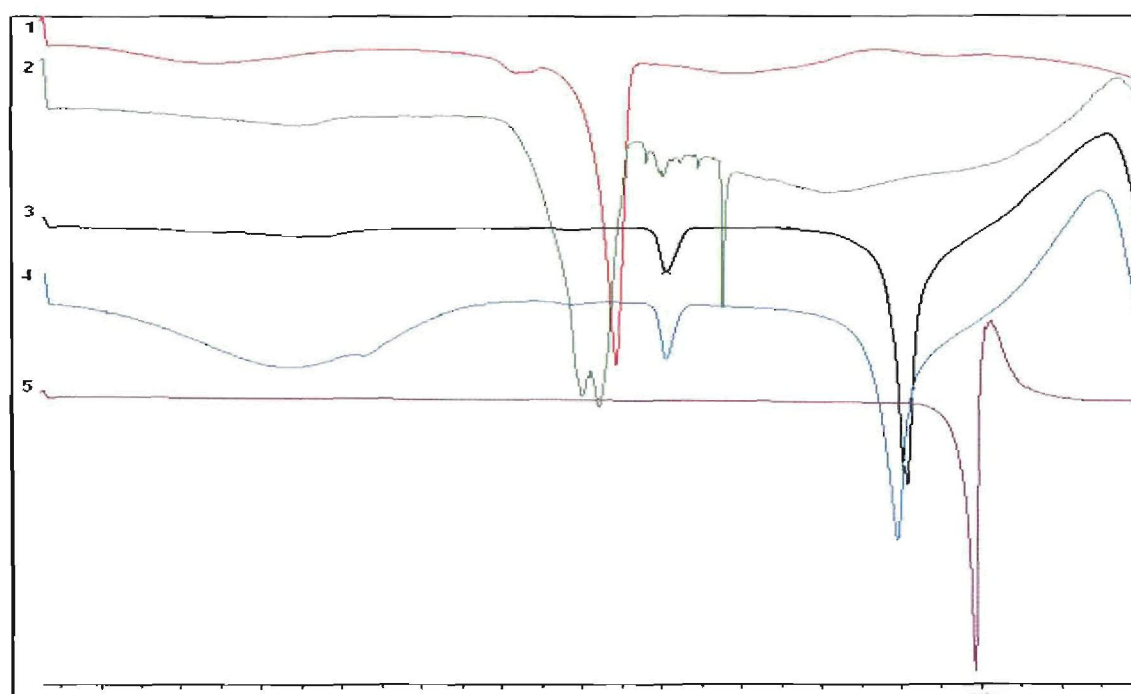


Figure 4.1: DSC Chromatograms of (1) capsule blend, (2) codeine phosphate + paracetamol, (3) meloxicam + codeine phosphate, (4) codeine phosphate and (5) crospovidone and codeine phosphate

From the analysis of the thermograms it can be seen that codeine phosphate exhibits two endotherms at 181.22 °C and 240.05 °C. There seems to be no apparent reaction between codeine phosphate and meloxicam since there was no notable shift of the codeine phosphate thermograms in the mixture. This suggests that there is no incompatibility between meloxicam and codeine phosphate. There was however a notable change in melting behaviour of thermograms for the mixtures of codeine phosphate and paracetamol and that of codeine phosphate and cospovidone. There were appearances of new peaks and disappearance of some codeine phosphate peaks for the mixture with paracetamol. There was also a disappearance of codeine phosphate peaks for the cospovidone mixture. This change in thermal behaviour of the mixtures suggests that there is possible interaction or incompatibilities between codeine phosphate, paracetamol and cospovidone. DSC predicts several interactions; however, it can not be concluded that the observed thermal events are detrimental nor that the combination with the ingredients involved should be avoided.

According to Botha *et al.* (1987:347), the interpretation of the thermal data is not always straightforward i.e. when two substances are mixed the purity of each is reduced and generally slightly lower melting points result. On the other hand, a large shift in melting point signifies that a strong solid-solid interaction has occurred. Nonetheless, DSC screening for compatibility does provide additional knowledge concerning possible interactions.

4.4.2 Potential polymorphism

In his thermal characterisation, Lahu *et al.* (1995:207) reported that codeine phosphate and codeine exist in a number of polymorphic forms which may influence its bio-availability. Lund (1994:815), reported that codeine exist in three polymorphic forms. Form I and Form II are the hydrous and anhydrous forms respectively and Form III is a solvate. Three metastable forms of paracetamol are known. Orthorhombic acetaminophen (paracetamol) which is suitable for direct compression tabletting and may also be slightly more soluble but has been crystallised only in small quantities. The only commercially available form is monocyclic acetaminophen, the thermodynamically most stable modification (Kalantzi *et al.*, 2006:5). Luger (1996:175) reported that under X-ray

crystallography meloxicam can be crystallised in four different prototropic forms: the anion, the acidic enolic form (suitable for pharmaceutical products), the zwitterion and the cationic form.

4.4.3 Dissolution for sparingly water soluble drugs

From Table 4.1 it can be seen that meloxicam has poor solubility characteristics in water, i.e. practically insoluble, thus the choice of the dissolution media for product release and development purposes needs to be carefully considered with the addition of a surfactant. The term surfactant is a convenient contraction for surface active agents. Surfactants play a major role in the absorption of drugs from the body. Because of the unique characteristics of surfactants, small concentrations added to water will immediately form a stable mono-layer. *In vivo*, surfactants are available to solubilise drugs. Drug release is usually the rate limiting process for absorption of low solubility oral drugs. Both *in vivo* physiology and the physico-chemical characteristics of the drug are important in the absorption of poorly water-soluble drugs.

4.4.3.1 Dissolution test for sparingly water soluble drugs

The United States Food and Drug Administration (FDA) has previously evaluated the effect of naturally occurring surfactants and compared them to commercially occurring surfactants. Because of the physiological relevance of surfactants, the FDA generally supports their use in dissolution testing of products with sparingly water-soluble drug. Naturally occurring surfactants solubilise sparingly-soluble drugs in the body and help in the absorption process. The use of a surfactant in the dissolution medium is physiologically relevant and can be successfully used for dissolution testing. In most cases sodium lauryl sulphate (SLS) has been used successfully (Noory, 2000:1-4).

In vitro test conditions should simulate the physiological conditions of the gastrointestinal tract where generally so-called “sink conditions” can be seen. The term is defined as the medium at least greater than three times that required for forming a saturated substance. The selection of a medium was based on the solubility data and the dose range of the product to ensure that the sink conditions were met. In addition the choice of surfactant,

agitation speed, apparatus and sampling time were primarily based on existing methods for the active pharmaceutical ingredients in the FDC capsule.

4.4.4 Formulation development

The developed product should be a conventional release oral dosage form. During the development phase the following laboratory trials were manufactured:

Tablets

PVP K30
Paracetamol dense powder
Codeine phosphate
Meloxicam
Lactose
Microcrystalline cellulose
Explotab
Pregelatinised starch
Sodium lauryl sulphate
Magnesium stearate
Opagloss red
Opagloss clear

Capsules

PVP K30
Paracetamol dense powder
Codeine phosphate
Meloxicam
Lactose
Microcrystalline cellulose
Crospovidone
Pregelatinised starch
Sodium lauryl sulphate
Colloidal silicon dioxide
Magnesium stearate
Orange and white gelatin capsules

The paracetamol and codeine phosphate used during the product development and the subsequent production batches was obtained from Fine Chemicals. Meloxicam was obtained from Denvados Quimco SA.

Direct compression (DC) formulations for the tablets were first explored but these were unsuccessful, thus a traditional granulation route was adopted. The tablets were caplet shaped and pale yellow in colour. These were the coated with Opagloss red to give them the red colour with a glossy finish due to the Opagloss clear. The reason for the clear coating was due to previously observed fading of the red colour by the supplier when Opagloss red was used on its own.

The granules used for filling capsules were also granulated in the same method as the tablets. The granules were pale yellow in colour and these were encapsulated into the orange and white gelatin capsules.

These trials were placed on short term stability for 6 weeks and 3 months at 25 °C and 60 % Relative humidity (RH). The tablets were also placed in a forced degradation incubator at 60 °C and 75 % RH for 4 weeks.

4.4.4.1 Results

Stability

The use of the red and clear coating solutions proved effective, as there was no apparent fade of the red colour. The results for these trials were satisfactory as there was no significant decrease in the assay of the actives or dissolution on stability with time. There was also no apparent decrease for actives for the samples subjected to forced degradation conditions after 4 weeks.

The tablet formulation was not pursued further as, although the coating of the laboratory scale trial was successful when using a smaller coating pan; when up scaling, coating proved to be problematic.

Dissolution

Various dissolution media employed during the development phase of the formulation included, 0.01 N HCl, phosphate buffer (pH 6.8 and 1.2), acetate buffer (pH 4.5) and 0.1, 0.15, 0.2 % and SLS in water. The development of the suitable dissolution media for meloxicam began with the solubility studies for meloxicam in different concentrations of sodium lauryl sulphate (SLS) due to meloxicam's low solubility. The tabulated experimental results of the dissolution study are shown in Table 4.3. The test volume was fixed at 900 ml.

Table 4.3: Solubility profiles of meloxicam in different dissolution media

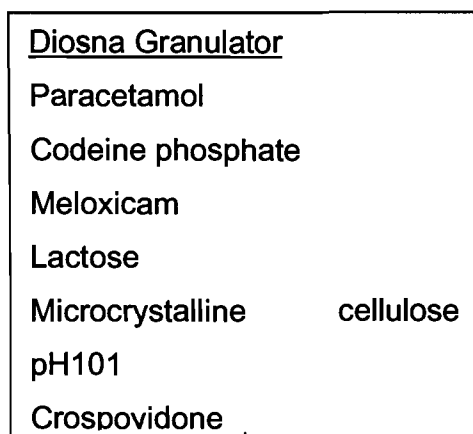
Dissolution Medium	% Dissolved			
	15 minutes	30 minutes	45 minutes	60 minutes
0.2 % SLS in Acetate buffer (pH 4.5)	65.09	84.58	97.90	105.74
0.05 % SLS	71.46	81.07	89.61	93.22
0.2 % SLS	82.89	93.81	97.13	97.93
0.15 % SLS	81.38	94.09	96.51	97.56
0.10 % SLS	55.80	85.74	92.54	93.75
0.2 % SLS in 0.01 N HCl (pH 1.2)	65.12	86.19	97.28	102.98
0.15 % SLS	84.14	93.20	96.86	98.36
0.1 % SLS	56.34	89.00	95.47	97.42
0.2 % SLS in Phosphate buffer (pH 6.8)	27.99	29.32	29.30	29.55
0.01 N HCl (pH 1.2)	85.99	95.40	97.00	97.26
0.1 % SLS in Phosphate buffer (pH 6.8)	N/A	91.86	94.16	97.06

Initially the phosphate buffer was prepared using potassium phosphate and this formed a precipitate when the SLS was added to the buffer solution. This effect was possibly due to the incompatibility of the potassium salt in the potassium phosphate and sodium salt in the SLS. The potassium phosphate was changed to a similar ion salt of SLS, sodium phosphate to prepare the buffer solution. No precipitation was seen with the use of the sodium salt. Thus the 0.1 % SLS in phosphate buffer (using sodium phosphate), was the medium of choice for meloxicam dissolution testing.

The method used fulfilled the sink conditions thereby avoiding saturation of the solution with the active ingredients, which may have lead to irreproducible and non-reliable dissolution results. The final choice of the media was further based on the USP first case where the 80 % of the labeled amount of meloxicam dissolved in 60 minutes using the paddle apparatus.

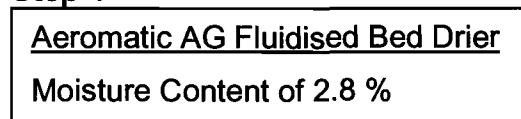
4.5 Manufacturing procedure flow diagram

Step 1



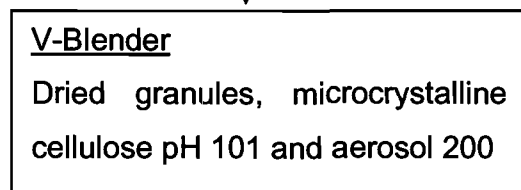
Dried at 60 °C

Step 4

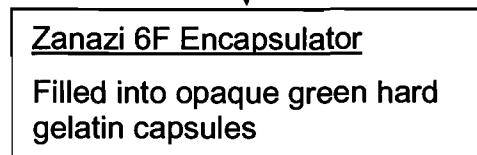


14 mesh (1.18 mm) sieve

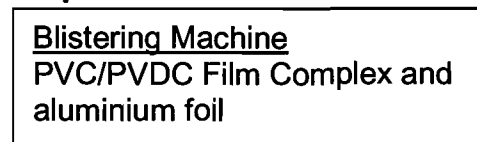
Step 5



Step 7



Step 8



Step 2

Dissolve sodium lauryl
sulphate in purified water
Dissolve polyvinylpyrrolidone
in water

Granulate

Step 3

Granulate to
suitable end-point

Purified water

Step 6

Magnesium stearate

In-process control checks
Capsule fill mass: 400.00 mg
Limits: (380.00–420.00) mg
Filled capsule length: 21.70 mm
Limits: (21.40 – 22.00) mm
Disintegration: NMT 30 minutes

In-process control checks
Leak test

4.6 Stability programme

The stability of a product relates to its resistance to various chemical, physical and microbiological reactions that may change the original properties of the preparation. Excipients can accelerate the existing instabilities of a drug substance or entirely new problems may arise due to chemical incompatibility between the excipient and the drug. The true role of an excipient is to produce a stable uniform product of high quality and to deliver the drug to the desired location at the desired rate (Letsoalo, 2001:20). The packed samples were placed onto a stability programme as per International Conference on Harmonisation (ICH) Guideline at 25 °C and 60 % Relative Humidity (RH), 30 °C and 65 % RH and 40 °C and 75 % RH. The samples placed on the stability programme were tested after 3, 6 and 9 months using the methods described in Chapter 2.

4.7 Stability programme results

The detailed stability results given in this chapter are that of the Triple FDC capsules. The Double FDC capsules were tested similarly to the Triple FDC capsules except for the tests related to meloxicam as they do not contain meloxicam. All the stability specifications for the Double FDC capsules were within limits except for the codeine phosphate assay. The placebo was tested for description, odour of contents, disintegration, average fill mass and loss on drying. There were no significant changes on stability compared to the initial testing and all specifications complied with the specified limits.

4.7.1 Description

The description of the granules i.e. pale yellow, odourless granules in an opaque dark green hard gelatin capsule did not change throughout the stability study.

4.7.2 Disintegration

There was no significant change in the disintegration time for the capsules over time nor at different temperatures. The disintegration time complied with the specification of not more than 30 minutes.

4.7.3 Average fill mass

The fill mass of the capsules was within the specified limits and there was no significant change in the fill mass of the capsules with time.

4.7.4 Uniformity of mass

All the capsules tested for uniformity of mass at each take off were within the specified BP limit.

4.7.5 Uniformity of content for meloxicam (initial testing only)

The meloxicam assay of all 10 capsules was between 85 to 115 %.

4.7.6 Loss on drying

The percentage loss on drying is given in Tables 4.4, 4.5 and 4.6. There was an increase in the percentage loss on drying with time on stability for capsules stored in each of the temperatures. However, this increase still falls within the specified BP limits of not more than 5 % m/m of its original weight.

4.7.7 Assay

The results given in Tables 4.4, 4.5 and 4.6 show that there were no significant changes in the percentage concentration for paracetamol and meloxicam over time and increase in temperature. However, there was a significant decrease of the codeine phosphate assay on stability with time. Surprisingly, this decrease was observed to a less effect with an increase in temperature and humidity.

4.7.8 Degradation products

The degradation products, 4-aminophenol (for paracetamol), 2-amino-5-methylthiazole and benzothiazine ethyl ester (for meloxicam) were not detected during the stability study. Codeine N-oxide and codeinone the degradation products of codeine phosphate were not tested during stability due to the previous history of satisfactory stability of codeine phosphate in finished product that Adcock Ingram has on a number of products previously developed. The degradation products were also not available from the approved suppliers. When the decrease of codeine phosphate was observed on stability, attempts

were made to import the degradation product codeine N-oxide and codeinone which are classified as Narcotics by the Department of Health in South Africa. The attempts made to procure the degradation products were unsuccessful as they were not available from the suppliers at that time. The degradation products for codeine phosphate were therefore not tested.

Table 4.4: Stability results for the Triple FDC capsules stored at 25 °C / 60 % RH

Tests	Limit	Initial	3 months	6 months	9 months
Physicals					
Disintegration	NMT	3 minutes	4 minutes	3 minutes	3 minutes
	30 minutes	51seconds	4 seconds	32 seconds	42 seconds
Loss on drying	NMT 5.0 %	0.5	0.5	0.9	1.3
Assay					
Paracetamol	250.0 mg	246.1 mg	254.3 mg	249.6 mg	249.5 mg
	237.5 – 262 mg	(98.44 %)	(101.73 %)	(99.85 %)	(99.79 %)
Codeine	10.0 mg	9.91 mg	8.80 mg	8.40 mg	8.47 mg
phosphate	(9.5 - 10.5) mg	(99.06 %)	(87.99 %)	(84.01%)	(84.69 %)
Meloxicam	2.5 mg	2.518 mg	2.411 mg	2.576 mg	2.576 mg
	(2.25 - 2.75) mg	(100.73 %)	(96.45 %)	(103.06 %)	(103.05 %)
Dissolution					
Paracetamol	10 minutes	92.35	87.49	84.28	82.38
% Dissolved in	15 minutes	97.50	96.31	96.83	94.10
	30 minutes	100.19	98.27	100.62	98.80
	(NLT 80 %)				
Codeine	10 minutes	98.47	78.18	75.90	78.71
phosphate	15 minutes	99.64	84.90	84.29	84.29
	30 minutes	99.86	85.30	85.10	86.24
% Dissolved in	(NLT 80 %)				
Meloxicam	30 minutes	85.93	91.86	84.81	82.27
% Dissolved in	45 minutes	94.18	94.45	93.84	92.04
	60 minutes	97.25	97.36	97.41	98.32
	(NLT 80 %)				

Table 4.5: Stability results for Triple FDC capsules stored at 30 °C / 65 RH%

Tests	Limits	Initial	3 months	6 months	9 months
Physicals					
Disintegration	NMT 30 minutes	3 minutes	4 minutes	3 minutes	3 minutes
		51 seconds	4 seconds	32seconds	42 seconds
Loss on drying	NMT 5.0 %	0.5	0.6	0.8	1.4
Assay					
Paracetamol	250.0 mg	246.1 mg	259.2 mg	252.8 mg	251.4 mg
	237.5–262.5 mg	(98.44 %)	(103.69 %)	(101.12 %)	(100.54 %)
Codeine phosphate	10.0 mg	9.91 mg	8.95 mg	8.89 mg	8.82 mg
	(9.5 - 10.5) mg	(99.06 %)	(89.46 %)	(88.89 %)	(88.18 %)
Meloxicam	2.5 mg	2.518 mg	2.493 mg	2.590 mg	2.637 mg
	(2.25 - 2.75) mg	(100.73 %)	(99.70 %)	(103.61 %)	(105.46 %)
Dissolution					
Paracetamol	10 minutes	92.35	85.14	91.02	87.69
% Dissolved in	15 minutes	97.50	93.98	96.02	93.40
	30 minutes	100.19	97.11	100.47	98.35
	(NLT 80 %)				
Codeine	10 minutes	98.47	79.37	86.60	85.54
phosphate	15 minutes	99.64	86.20	88.05	87.76
% Dissolved in	30 minutes	99.86	87.02	89.87	88.59
	(NLT 80 %)				
Meloxicam	30 minutes	85.93	74.88	76.65	79.57
% Dissolved in	45 minutes	94.18	88.27	89.95	89.44
	60 minutes	97.25	92.94	95.21	94.76
	(NLT 80 %)				

Table 4.6: Stability results for capsules stored at 40 °C / 75 % RH

Tests	Limits	Initial	3 months
Physicals			
Disintegration	NMT 30 minutes	3 minutes 51 seconds	3 minutes 42 seconds
Loss on drying	NMT 5.0 % m/m	0.5	0.8
Assay			
Paracetamol	250.0 mg (237.5 – 262.5) mg	246.1 mg (98.44 %)	255.0 mg (102.02 %)
Codeine phosphate	10.0 mg (9.5 - 10.5) mg	9.91 mg (99.06 %)	9.27 mg (92.71 %)
Meloxicam	2.5 mg (2.25 - 2.75) mg	2.518 mg (100.73 %)	2.485 mg (99.40 %)
Dissolution			
Paracetamol	10 minutes	92.35	82.74
% Dissolved in	15 minutes	97.50	90.86
	30 minutes (NLT 80 %)	100.19	97.39
Codeine phosphate	10 minutes	98.47	88.67
% Dissolved in	15 minutes	99.64	91.92
	30 minutes (NLT 80 %)	99.86	93.04
Meloxicam	30 minutes	85.93	64.17
% Dissolved in	45 minutes	94.18	77.63
	60 minutes (NLT 80 %)	97.25	85.93

4.7.9 Dissolution

The *in vitro* release profiles of the active ingredients from the Triple FDC capsules are depicted in Figures 4.2, 4.3, 4.4, 4.5, 4.6 and 4.7. The dissolution rate was measured in 0.01N HCl medium for paracetamol and codeine phosphate and in phosphate buffer pH 6.8 with 0.1 % SLS for meloxicam. Dissolution was conducted using the method described in Chapter 2. The dissolution was within the specified limits for all the three actives. However, the dissolution rate for meloxicam slowed down significantly over time

with the increase in temperature when compared to the initial dissolution rate. The same effect was observed with codeine phosphate to a lesser extent and although the amount dissolved in 45 minutes was within the specified limit it became less available with time. Although there was a decrease in dissolution rate, all three actives complied with the requirements for dissolution whilst on stability. The decreased availability of the codeine phosphate with time correlates to the decreased assay of codeine phosphate on stability.

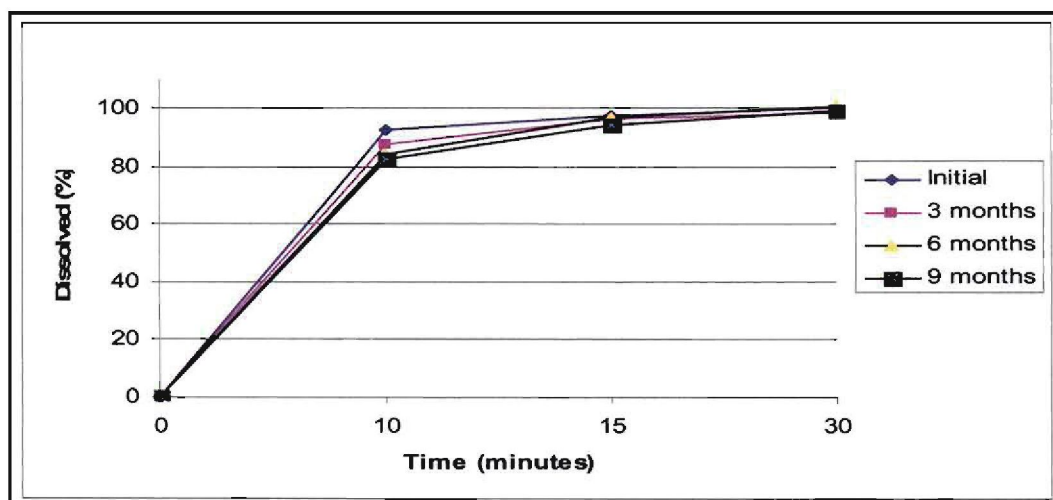


Figure 4.2: Dissolution profiles of paracetamol stored at 25 °C and 60 % RH

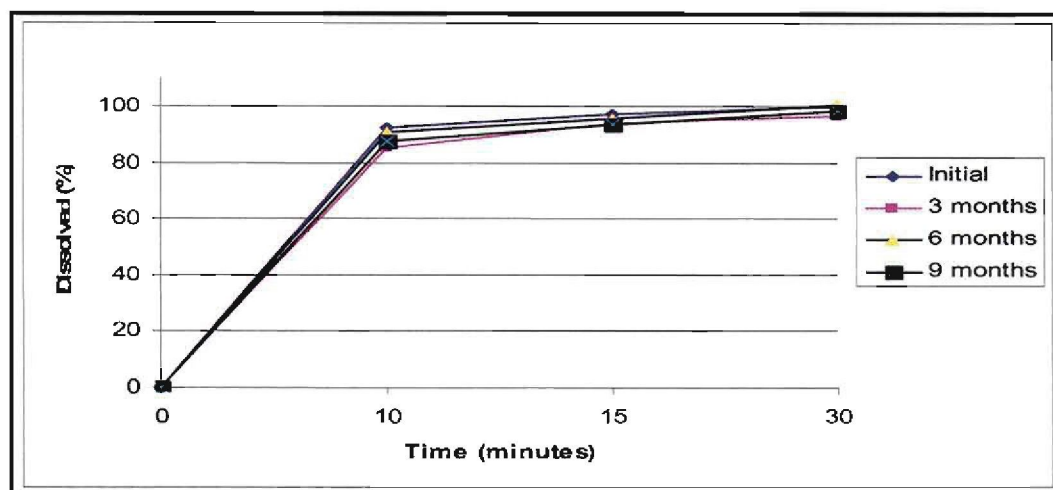


Figure 4.3: Dissolution profiles of paracetamol stored at 30 °C and 65 % RH

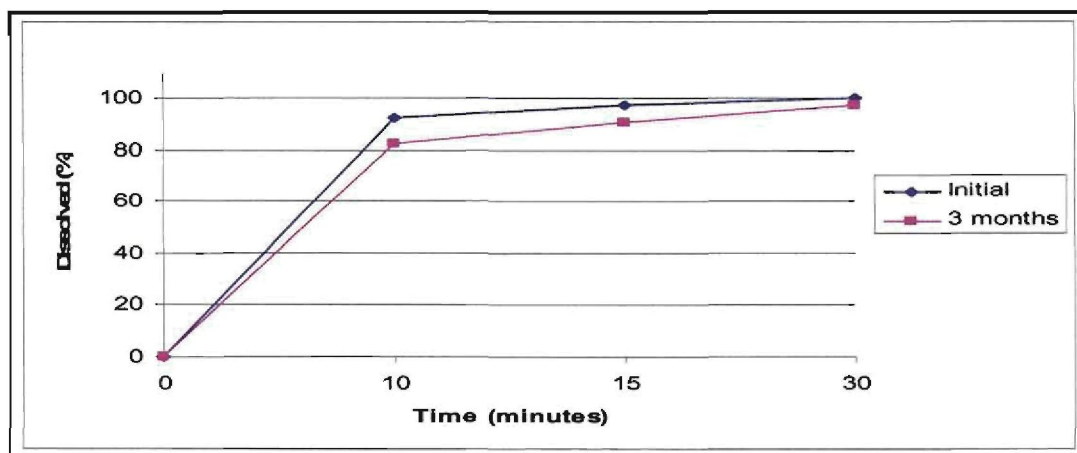


Figure 4.4: Dissolution profiles of paracetamol stored at 40 °C and 75 % RH

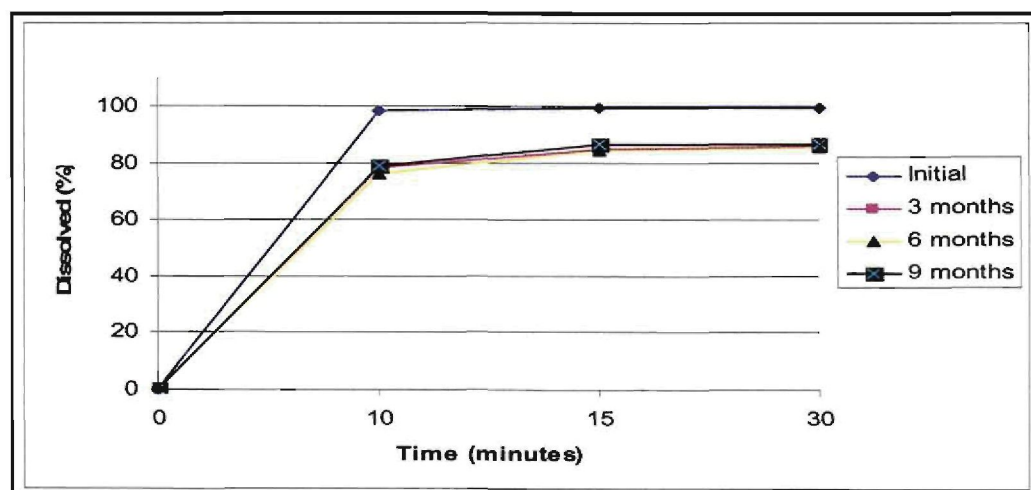


Figure 4.5: Dissolution profiles of codeine phosphate stored at 25 °C and 60 % RH

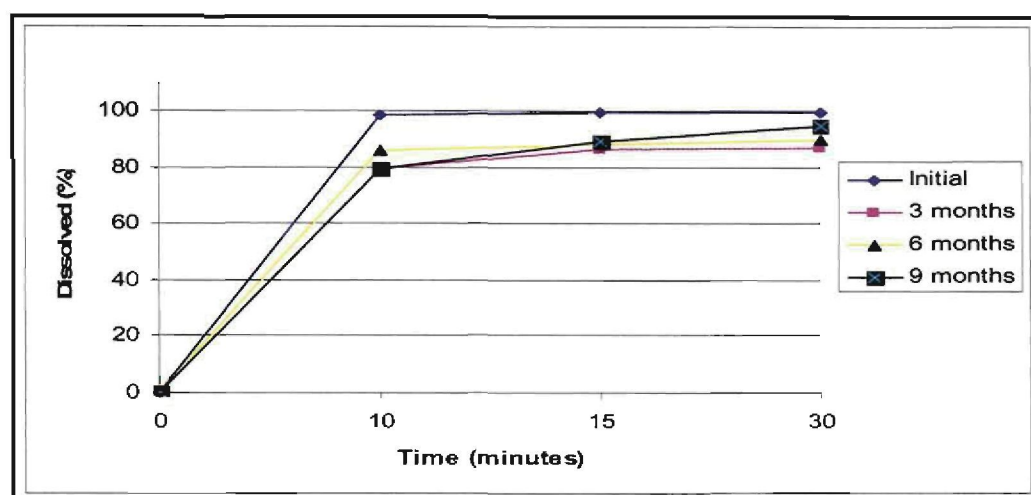


Figure 4.6: Dissolution profiles of codeine phosphate stored at 30 °C/65 % RH

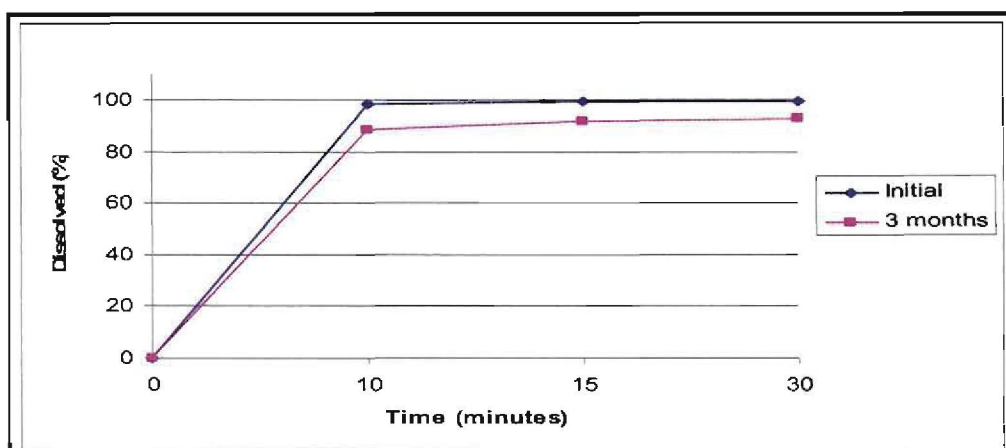


Figure 4.7: Dissolution profiles of codeine phosphate stored at 40 °C and 75 % RH

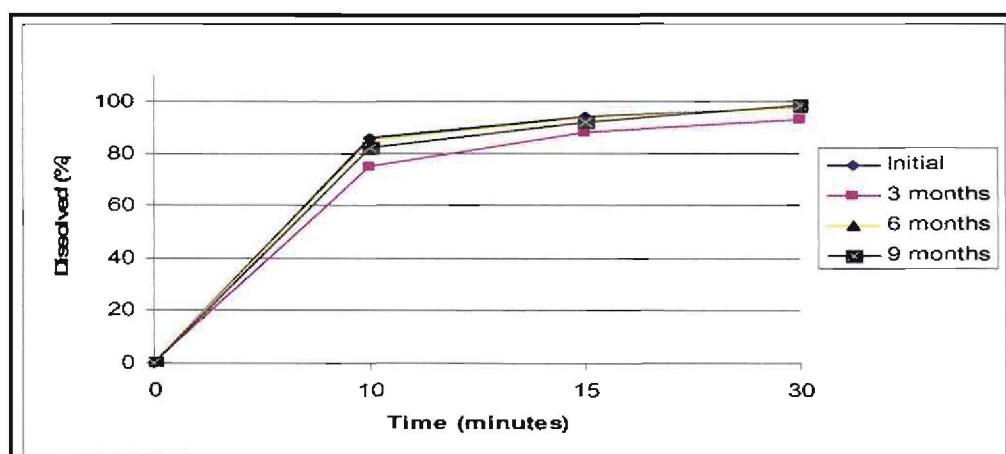


Figure 4.8: Dissolution profiles of meloxicam stored at 25 °C and 60 % RH

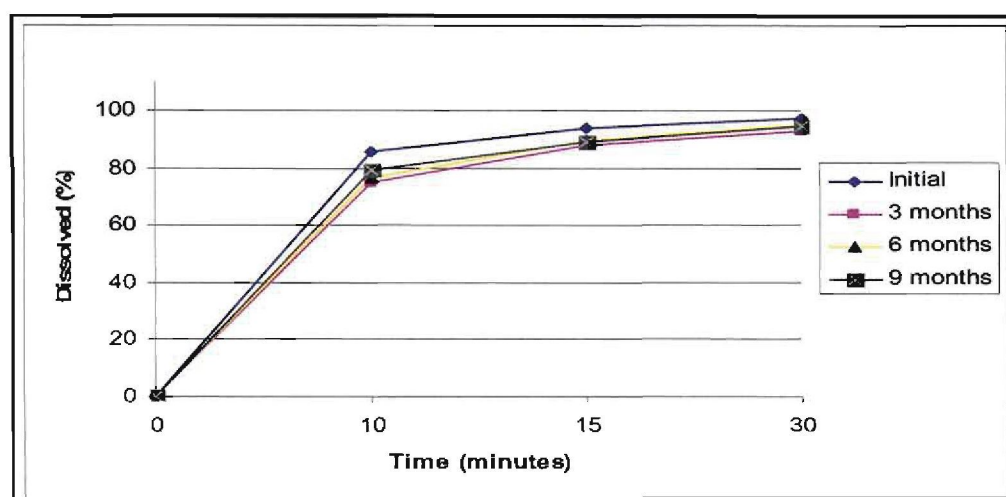


Figure 4.9: Dissolution profiles of meloxicam stored at 30 °C and 65 % RH

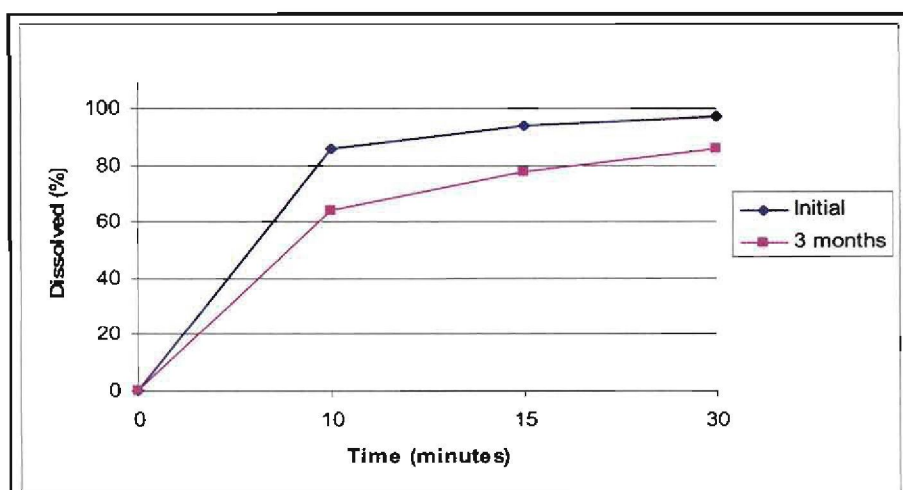


Figure 4.10: Dissolution profiles of meloxicam stored at 40 °C and 75 % RH

4.8 Discussions

Pellerin *et al.* (1964:500) reported that the possibility of an interaction between codeine phosphate and sodium lauryl sulphate was between 8 – 15 %. Further investigations were carried out to confirm whether Pellerin finding could actually be the reason why codeine phosphate assay decreased on stability. The initial blend (not capsulated) used for encapsulation into the green hard gelatin capsules and the laboratory trial batch of the filled white and orange capsule were re-assayed. These were stored in ambient temperature in securitainers in a cupboard.

The results showed that there was no change in the assay amount of codeine phosphate even though the initial blend and the orange and white capsules were over 6 months. At the time of this analysis, the decreased codeine phosphate in the green gelatin capsules was after 3 months stability testing period, less than the samples that were stable. Laboratory trial: 96.56 %

Initial capsule blend: 100.29 %

At the time of this analysis, the decreased codeine phosphate in the green gelatin capsules was after 3 months stability testing period, less than the samples that were stable. From these results it could be observed that there doesn't seem to be any reaction between SLS

and codeine phosphate that could lead to a decrease in codeine phosphate for these products. The only difference between the samples where codeine phosphate was still within specification and those where codeine phosphate has decreased is the colour of the gelatin capsule shell. It is thus suspected that the reason for the out of specification results could be due to the colourant in the green capsule shell and not the SLS as initially postulated. The decreased results due to the incompatibility of the green capsule shell and codeine phosphate; over time was not be picked up during method validation. The codeine phosphate and the green capsule shell could be forming a complex or an interaction may be taking place between the two. The exact nature of the interaction could not be ascertained.

4.9 Conclusion

The products developed complied with all the requirements for initial analysis before being encapsulated and placed on stability for both the Triple and the Double FDC. On stability, the codeine phosphate assay and dissolution for both the Triple FDC and Double FDC capsules decreased significantly with time. The decreased codeine phosphate results during dissolution testing correlates to the assay results obtained on stability. Although there was a decrease in the dissolution rate of codeine phosphate, the percentage dissolved was still within the specified limit of not less than 80 % dissolved in 60 minutes. The changes for the other parameters were not significant and complied with the specified limits.

This decrease was also seen with the Double FDC. It is suspected that the decrease in codeine phosphate assay on stability is due to a complex formation or incompatibilities between codeine phosphate and the capsule shell used. The exact mechanism of the suspected incompatibility or complex formation could not be ascertained.

CHAPTER 5

BIOAVAILABILITY STUDY

INTRODUCTION

This study focuses on the pharmacokinetic properties of the Triple fixed dose combination (FDC) capsules. The aim of the study was to establish whether meloxicam altered the pharmacokinetics of paracetamol and codeine phosphate in a fixed-dose combination of all three actives, and whether paracetamol and codeine phosphate altered the pharmacokinetics of meloxicam in a fixed-dose combination capsule. The bioavailabilities of paracetamol, codeine phosphate and meloxicam from Triple FDC capsules were compared with those from Myprodol® (paracetamol and codeine only) and Mobic® (meloxicam). Myprodol®, was chosen as reference product because it was the only commercially available fixed-dose combination which contains paracetamol and codeine phosphate in the required configuration at the time when the study commenced. Myprodol® contains ibuprofen which did not form part of the comparative analyses. Mobic® (7.5 mg meloxicam) is the South African innovator product and thus was used as a reference product.

Carrasco-Portugal (2005:307-313) reported inter-ethnic differences in the pharmacokinetics of meloxicam and Jung (2004:1037) reported those of codeine phosphate. To minimise possible inter subject variability, only caucasians were enrolled in this study.

5.1 Study objective

A study to determine the bioavailability of the three components of Triple FDC (paracetamol, codeine phosphate and meloxicam) relative to Myprodol® (paracetamol, ibuprofen and codeine phosphate) and Mobic® (meloxicam), in a single dose, laboratory-blind, randomized, three-part crossover design in healthy male and female caucasian volunteers, a Phase I study was conducted. There was a drug-free period of at least 14 days between product administrations. The study was conducted at AddClin Research (Pty) Ltd, Pretoria, South Africa.

5.1.1 Independent Ethics Committee and Local Regulatory Authorities

The study protocol, consent form and the volunteer information leaflet were reviewed and approved by an Independent Ethics Committee (IEC), Pharma-Ethics Independent Research Ethics Committee. The South African Medicines Control Council (MCC) also reviewed and approved the protocol, and authorised the use of the investigational products for the duration of the study.

A written approval of the study protocol from the Pharma-Ethics Independent Research Ethics Committee and the MCC was obtained before initiation of screening procedures. This study was conducted in accordance with International Conference on Harmonisation Guidelines on Good Clinical Practice for clinical studies conducted outside the European Union region.

5.1.2 Ethical conduct of the study

This study was conducted, and informed consent obtained, according to the ethical principles stated in the Declaration of Helsinki (52nd WMA General Assembly,) and the ICH Guideline for Good Clinical Practice (1997) (GCP).

The conduct of the study complied with the following recommendations and guidelines:

- Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa, 2000.
- The Declaration of Helsinki (52nd WMA General Assembly).
- The ICH Guideline for Good Clinical Practice, (ICH Topic 6, 1997).

5.1.3 Volunteer information and Informed consent

Before commencement of the study, the investigators explained to all volunteers the nature, purpose and risks of the screening procedures, as well as the nature, purpose, obligations, restrictions and risks relevant to the study, both orally and in writing. This included the study aims, the expected duration, expected benefits for others, the reference treatment, the steps which were to be taken and the requirements from the volunteers, the risks and inconveniences of participation, the undesirable effects or hazards of the investigational products, the number of participants, a contact name should they require any additional information and the reimbursement for inconvenience. Volunteers were informed that they were not in any way obliged to participate and they were given ample

opportunity to inquire about details of the study. It was made clear that refusal to participate or withdrawal from the study at any stage was without any disadvantage to their subsequent care.

Prior to participation in the trial, the written informed consent form was signed and personally dated by the volunteer and by the person who conducted the informed consent discussion (either investigator or designee and the investigator). The volunteer received a copy of the signed and dated informed consent form. As part of the consent process, each volunteer consented to direct access to his medical records for trial-related monitoring, auditing, IEC review, and regulatory inspection. Volunteer information leaflets regarding the conduct of the study and the informed consent form were available in English and Afrikaans. Each volunteer was provided with a copy of this form and the informed consent form in the language of his or her choice.

5.2 Study design

5.2.1 Study population

5.2.1.1 Sample size

Twenty-four eligible male and female volunteers were enrolled to ensure that a minimum of 21 volunteers completed the study. Information gathered would suffice to predict future bioequivalence sample sizes since this study focused mainly on bioavailability and not bioequivalence.

5.2.1.2 Inclusion and Exclusion Criteria

The inclusion and exclusion criteria were set to ensure a homogeneous, healthy study population that would facilitate the conduct and scientific evaluation of the study. The specific criteria were also selected to minimise the risk to the well-being of the volunteers. Volunteers were required to comply with the following inclusion and exclusion criteria to be eligible for the study.

Inclusion Criteria

The volunteer:

- is a caucasian male or female and aged between 18 and 45 years, inclusive.

body weight must be within 15 % of the ideal weight according to the body mass index (BMI).

- is in general good health, as determined by a medical history, physical examination, 12-lead ECG, blood pressure, pulse rate, oral temperature and clinical laboratory results within the “normal” ranges for the relevant tests (unless the clinical investigator considers the deviation to be irrelevant for the purpose of the study), e.g. congenital non-heamolytic hyperbilirubinaemia.
- smokes less than 10 cigarettes/pipes per day and is willing to abstain from smoking for the duration of each admission period.
- Female volunteers are using appropriate contraception, i.e. an oral contraceptive for at least 6 months prior to entry into the study and an oral contraceptive or barrier method plus spermicide during the study and for at least 3 months after study completion.

Exclusion Criteria

The volunteer has:

- evidence or history of clinically relevant psychiatric, hepatic, renal, respiratory, cardiovascular, gastrointestinal, endocrine or central nervous system disease.
- had a major illness during the 3 months before commencement of the study-related procedures.
- a clinically relevant physical or organ abnormality.
- a resting (after 5 minutes) supine diastolic blood pressure greater than 90 mmHg or less than 60 mmHg, or a supine systolic blood pressure greater than 150 mmHg or less than 100 mmHg.
- a systolic blood pressure decrease of 20 mmHg or more or a diastolic blood pressure decrease of 10 mmHg or more, between supine and 3 minute after standing in an orthostatic blood pressure assessment.
- a resting (after 5 minutes) supine pulse rate less than 50 beats per minute (bpm) or greater than 100 bpm.
- clinically relevant out-of-range values for haematology, serum chemistry or urine analysis parameters (ALT, AST and GGT values more than twice the upper limit of the normal range are regarded as clinically significant).

- a history of hypersensitivity to any drugs.
- a history of any severe allergies.
- is known to be HIV1/2Ab, HBsAg or HCV-Ab positive.
- participated in another study with any investigational product within 8 weeks before the first administration of the current investigational products, or until at least 5 x half-life ($t_{1/2}$) elimination has lapsed, whichever is the greater.
- received treatment within the previous 3 months with any drug having a well-defined potential for adversely affecting a major organ or body system at the recommended doses.
- a history of or current misuse of substance of abuse/medication.
- positive urine screen for drugs of abuse
- a history of or current compulsive alcohol abuse (> 28 units per week, one unit is equivalent to 8 - 10 g of ethanol), or has a positive result for alcohol from a saliva sample.
- made a blood donation or had a comparable blood loss (500 ml) during the 8 weeks before administration of the first investigational product (IP).
- a history of bronchial asthma.
- females of childbearing potential who are pregnant, planning to become pregnant, breastfeeding or not using acceptable birth control.

5.2.2 General and dietary restrictions on volunteers

5.2.2.1 Diet

The volunteers would fast for 5 hours before attending their examinations at screening. The ingestion of food and beverages containing alcohol, caffeine or grapefruit would not be permitted for 24 hours prior to investigational product (IP) administration and for the duration of each study period. Food and fluid intake was regulated for the duration of the admission periods.

5.2.2.2 Activity

Strenuous activity e.g. strenuous gym sessions were not allowed for 24 hours before the screening examination and from 24 hours before each IP administration and for the duration of each study period (i.e. until after collection of the 96 hours post-dose blood sample). Volunteers would remain in a semi-recumbent position after dosing until lunch is

served at 5 hours after dosing except for visits to the lavatories. After that, posture and activity would not be restricted but no strenuous activity was allowed as detailed previously.

5.2.3 Medication

The IPs were administered as prescribed in paragraph 5.4.2. Volunteers refrained from using any prescribed medicines and over the counter medications for 7 days before the first IP administration until completion of the study. If any medications other than the prescribed IPs were required or indicated, the investigator was contacted immediately to discuss the most appropriate action before taking any medicines. The volunteer's continued participation in the study was assessed based on the time-dependant pharmacodynamic and pharmacokinetic properties of such medicines relative to the time the medication was administered and the possible influence of the ingested medication on the pharmacokinetics of the IPs, interference with the assay method and well-being of the volunteer.

5.3 Investigational products

5.3.1 Reference product

5.3.1.1 Treatment A

Trade name: Mobic® 7.5 mg tablet
Manufacturer: Boehringer Ingelheim (Pty) Ltd, South Africa
Dosage form: Tablet containing 7.5 mg meloxicam per tablet for oral administration
Dose: A single dose of 7.5 mg meloxicam (1 tablet)

Dose: A single dose of 600 mg ibuprofen, 750 mg paracetamol and 30 mg codeine phosphate (3 capsules)

5.3.1.2 Treatment B

Trade name: Myprodol® capsules
Manufacturer: Adcock Ingram Limited , South Africa
Dosage form: Capsule containing 200 mg ibuprofen, 250 mg paracetamol and 10 mg codeine phosphate per capsule for oral administration

5.3.2 Test product

5.3.2.1 Treatment C

Approved name:	Triple FDC capsules
Dosage form:	Capsule containing 2.5 mg meloxicam, 250 mg paracetamol and 10 mg codeine phosphate per capsule for oral administration
Dose:	A single dose of 7.5 mg meloxicam, 750 mg paracetamol and 30 mg codeine phosphate (3 capsules)

5.3.3 Treatments and dosages

Volunteers were assigned to a treatment order according to the randomisation schedule. Volunteers were to receive each treatment once.

5.3.4 Analysis of IPs

The IPs were accompanied by certificates of analysis (test product) before commencement of the study. The IPs were coded and labelled in a manner that protected the blinding.

5.4 Investigational plan

5.4.1 Screening procedures

Potential participants were examined within the fourteen days prior to the first administration of the IPs to assess their eligibility to participate in the study. They had been instructed to fast for 5 hours before attending their examinations, ethical and regulatory aspects. The screening examinations and investigations included:

- Medical history, demographics, physical examination, alcohol and tobacco consumption patterns.
- Measurements of height and weight for the calculation of the BMI.
- Vital signs: pulse, systolic and diastolic blood pressure in supine (after 5 minutes rest) and thereafter standing positions (3 minute) and body temperature.
- Standard 12-lead ECG (was recorded in the supine position after a 5 minute resting period in the supine position).

- Haematology, serum biochemistry and urine analysis.
- Urine screen for drugs of abuse.
- Alcohol saliva test.
- Urine pregnancy test (except volunteers who have had hysterectomies).

Results of pre-study laboratory investigations indicating values for one or more variables outside the normal ranges would not necessarily lead to exclusion of a volunteer from the study. They would be interpreted in the clinical context. The investigator would assess the relevance of each laboratory value (at screening and post-study) outside the normal ranges. He or she would write NCR (not clinically relevant) or CR (clinically relevant) on the printed laboratory report next to each value outside the normal range, initialing each one. The investigator would sign and date each results page of the report to confirm that the report has been reviewed. The investigator would record any clinically relevant abnormal value in the Case report forms (CRFs). Post-study laboratory investigations indicating values outside the normal ranges may be repeated to establish if and when those values return to within the normal ranges.

Values outside the normal ranges would, however, be reviewed in the clinical context and in regard to other relevant investigations and also in light of the specific IPs administered, before a decision is taken to repeat the investigation(s) in question. Any clinically relevant changes from screening that were observed post-study (including those where the post-study value remained within the normal range) would be recorded as adverse events (AEs) in the CRFs by the investigator.

5.4.2 Study periods

Volunteers were admitted to the research site at approximately 21H00 on the evening prior to administration of the IP. The investigator confirmed that volunteers were healthy and still eligible for dosing by conducting a brief health check to ascertain any AE experiences and/or medication/s used in the period between screening and admission. The extent of examination thereafter depended on this information. A urine pregnancy test was

performed (female volunteers) volunteers were asked how they were feeling and how they had been since they attended the research site for the screening examination. They were also asked whether they had abided by the dietary and activity restrictions (i.e. no alcohol- or caffeine-containing drinks or food and strenuous activity during the 14 hours prior to admission). They remained at the research site for approximately 12 hours after the IP administration during which time blood samples were collected. Volunteers fasted from 21H00. The admission safety assessments were completed and eligibility to continue was confirmed and recorded in the individual CRFs.

Volunteers were woken at approximately 05H00 on each dosing day. They were given 200 ml of tap water to drink upon awakening. After showering, vital signs and body temperature were measured. The investigator confirmed eligibility for dosing. An indwelling venous cannula was inserted into a forearm vein of each volunteer and a 10 ml pre-dose blood sample was drawn within 1 hour of drug administration. Shortly before dosing commenced volunteers were told to empty their bladders.

Following pre-dose assessments there were potential exclusions if the volunteers had:

- received a regular course of prescribed medication, during the 7 days before admission for the first study period, or until at least 5 times $t_{1/2}$ elimination has lapsed, whichever is the greater.
- taken any over the counter medication during the 7 days before the first IP administration, unless the investigator considers such self-medication not to be of clinical relevance to the study.
- consumed any alcohol or caffeine-containing products (i.e. tea, coffee, cola, chocolate) within 24 hours before the first dose administration.
- participated in strenuous exercise within 24 hours before the first IP administration.
- positive urine pregnancy test results.

At approximately 07H00 the volunteers swallowed the relevant investigational product with 200 ml tap water (whilst standing) as assigned according to the randomisation plan. For practical reasons, the administration of the IP was performed at staggered intervals (± 3 minutes apart). Compliance and correct dose administration and subsequent posture were

ensured. The actual time of drug administration was documented in the individual CRFs. Treatment was not blinded to volunteers nor the investigator team. The laboratory team remained blinded throughout the study.

5.4.3 Dosage instructions

To ensure compliance, the IPs were administered by a Research Pharmacist under supervision of an investigator. Mouth checks were done to ensure volunteers have swallowed their tablets or capsules. The actual time of IP administration was documented directly onto the CRF and the statistical analysis was done using the actual time that each blood sample was collected (actual blood sampling times relative to the actual time of drug administration).

Blood samples were collected at specified times after IP administration as described in paragraph 5.4.4. Volunteers remained in a semi-recumbent position until 5 hours after IP administration except for visits to the lavatories. From 5 hours after dosing, posture and movement was not restricted, with the exclusion of strenuous exercise. Volunteers did not leave the research site until discharged (after collection of the 12-hours post-dose sample).

Standardised meals and beverages were served at specific intervals after IP administration: lunch at approximately 5 hours post-dose and an afternoon snack at 8 hours post-dose. The meals were standardised with respect to content and quantity for all study periods. Volunteers drank 200 ml water at 2 and 4 hours after dosing. Decaffeinated beverages (240 ml) were provided with meals and snacks at 5 and 8 hours after dosing. Water was allowed *ad libitum* for thirst after lunch (5 hours post-dose). No other food and fluids were allowed during the admission period.

Just prior to collection of the 12-hour post-dose blood samples, volunteers were asked how they had felt since asked previously, any AEs were reported and the use of concomitant medication was fully documented. Confirmation of their fitness to be discharged depended on this information. Volunteers left the research site after collection of the 12-hour post-dose blood sample, provided they were fit to do so. Before discharge from the first study

period, each volunteer was given an identification card and instructed to keep it with them for the duration of the study and that, should the need arise for consultation with another clinician.

Volunteers returned to the research site in adequate time to provide the follow-up blood samples at 24, 48, 72 and 96-hours post-dose. During all subsequent visits, AEs, use of concomitant medication and compliance to study restrictions were checked and documented.

5.4.4 Blood sampling

Venous blood samples (10 ml each for 0 to 12-hour samples, 5 ml each for 24 to 96-hour samples) were collected into lithium heparinised polypropylene tubes according to the following time schedule:

Before IP administration (not more than 60 minutes pre-dose) and at 20, 40 minutes and at 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 24, 48, 72 and 96-hours post-dose.

Blood samples were collected through an indwelling venous cannula for as long as possible, and by venipuncture when applicable. The actual blood sampling times were documented directly into the CRFs.

5.4.4.1 Handling of samples: Special instructions

The blood collection tubes were inverted gently a few times after collection and stored on ice until centrifuged at about 0 – 8 °C within 1 hour of collection, at about 2700 G for 10 minutes. The supernatant was divided into 6 aliquots containing as a minimum the volumes as specified below:

- one aliquot of 0.5 ml for analysis of paracetamol
- one duplicate back-up sample of 0.5 ml for paracetamol
- one aliquot of 0.5 ml for analysis of codeine
- one duplicate back-up sample of 0.5 ml for codeine
- one aliquot of 0.3 ml for analysis of meloxicam and
- one duplicate back-up sample of 0.3 ml for meloxicam.

The aliquots were transferred to the appropriately labelled polypropylene tubes using disposable pipettes. The labels were identical to those used on the appropriate blood collection tube. The samples were transferred immediately into “dry ice”, thereafter to a -20 °C freezer pending shipment to Farmovs-Parexel CRO Bioanalytical Laboratory where the samples were analysed. Samples were kept frozen during shipment by packing them in “dry ice”. The back-up aliquots were shipped to the analytical laboratory after safe arrival of the first set has been confirmed. All plasma samples were stored in -20 °C freezers.

5.4.4.2 Volume of blood samples

A total of 17 samples were collected per volunteer per study period; the blood volume per study period was 150 ml; the blood volume for pre- and post-study laboratory investigation was unlikely to exceed 50 ml. The total volume of blood sampled during the entire study was unlikely to exceed 500 ml.

5.4.4.3 Bio-analytical investigation

Quantitative analyses of meloxicam, paracetamol and codeine phosphate in the individual plasma samples and suitable quality control samples were performed by the Farmovs-Parexel CRO Bioanalytical Laboratory using LC/MS/MS validated methods described in Chapter 2.

Analyte 1 – Paracetamol

Name:	Paracetamol
Supplier:	Sigma-Aldrich
Batch number:	Lot 032K0146
Expiry date:	30 April 2007

Internal standard

Name:	Proxiphylline
Farmovs-Parexel pure substance number:	R395

Analyte 2 – Codeine

Name:	Codeine phosphate
Manufacturer:	Fine Chemicals Corporation
Batch number:	Batch CPFH84

Farmovs-Parexel pure substance number:	R166c
Expiry date:	August 2010

Internal standard

Name:	Naloxone
Farmovs-Parexel pure substance number:	R601

Analyte 3 – Meloxicam

Name:	Meloxicam
Supplier:	Derivados Quimicos S.A.
Batch number:	Batch 8010
Farmovs-Parexel pure substance number:	R145c
Expiry date:	31 December 2008

Internal standard

Name:	Piroxicam
Farmovs-Parexel pure substance number:	R050e

The individual plasma concentrations of meloxicam, paracetamol and codeine were tabulated for each volunteer, product and scheduled sampling time, together with descriptive statistics. Concentrations below the lower limit of quantification (LLOQ) are indicated by below the limit of quantification (BLQ). All BLQ values occurring after t_{max} and before the last quantifiable concentration were substituted by half the lower limit of quantification (LLOQ) value. For calculation of descriptive statistics of BLQ values, occurring before t_{max} were substituted by zero. These substitutions were made to allow the graphical representation of the mean plasma paracetamol, codeine and meloxicam for the test and reference products. Non-reportable or missing values were ignored in all calculations.

Meloxicam: $10.17\text{ng/ml}/2 = 5.09\text{ ng/ml}$

Paracetamol: $102.01\text{ng/ml}/2 = 51.01\text{ng/ml}$

Codeine phosphate: $1.58\text{ng/ml}/2 = 0.79\text{ng/ml}$

For the calculation of the terminal regression line, however, values below LLOQ, occurring before $t_{\max}$, were substituted with zero and values below LLOQ occurring after $t_{\max}$ were set to missing. The area under the plasma concentration-time curve was calculated using the linear trapezoidal rule. The slowest disposition rate constant (L_z), was calculated using WinNonlin® Pro version 5.0.1. The minimum number of data points used for the calculation of L_z was three. $C_{\max}$ was not included in the calculation of L_z . No half life was calculated if the minimum number of points available for the calculation of L_z was 2 or less, or if the value at the final data point above the limit of quantification was greater than the second last value above the limit of quantification.

Extrapolation of the area under the curve to infinity was carried out using linear regression on the logarithmic ($\ln$) transformed data points of the curve. The extrapolated area was calculated as the computed last value divided by L_z (C_T/L_z). No weighting was applied to the regression line. The slope of the line is equal to L_z . The drug half-life, $t_{1/2,z}$ was calculated as $\ln 2/L_z$.

AUC_{0-t} and $C_{\max}$ were subjected to an analysis of variance (Latin Square Design) with sequence, volunteer (sequence), product and period as the main effects for the logarithmic transformation of the data.

All pharmacokinetic calculations were performed using WinNonlin® Pro version 5.0.1. Statistical analyses were performed using SAS® Version 8.2. Individual values of the pharmacokinetic variables for paracetamol, codeine and meloxicam are listed with descriptive statistics tables, AUC_{0-t} and $C_{\max}$; $t_{\max}$, $t_{1/2,z}$, $AUC_{0-\infty}$, and $MRT_{0-\infty}$.

5.4.5 Post-study follow-up

The post-study safety evaluation was done after collection of the 96-hour post-dose sample of the third study period or, in the case of a drop-out, within 72 hours of withdrawal from the study. The evaluation included:

- A health check, including a physical examination and measurement of vital signs (supine blood pressure and pulse after resting for 5 minutes, standing blood pressure and pulse after standing for 3 minutes and body temperature).

- Review of any ongoing adverse events.
- Urine pregnancy test.
- Haematology, serum biochemistry and urine analysis.

5.5 Data management

All data management activities were performed by Farmovs-Parexel's Biometry Division. The system that was used for all these activities is iVal which is a validated, 21 CFR Part 11 compliant application.

Independent, double-data entry onto an electronic database was performed by two different data capturers. Verification of the data was performed after a comparison of the two entries. Validation checks were programmed for the study and were run after the second data entry was completed. Irresolvable inconsistencies were referred to the investigational team for clarification. Upon completion of all the validation processes, and after a statistical review of the data, the database was "locked".

Data was extracted from the database directly onto data files for statistical analyses. Modifications and updates to the database were documented in an audit trail. Medical and surgical history data were not coded. Adverse event data were coded using MedDRA version 9.1. Previous and concomitant medication taken at any stage during the study was coded using WHO-DD version 2006, quarter 1.

5.6 Safety variables

5.6.1 Recording of adverse events and concomitant medications

Occurrence of AEs was assessed throughout the study. The details of all AEs reported by volunteers or observed by the study personnel during the entire study, were recorded onto the appropriate source document, regardless of their causal relationship. Similarly, the use of all concomitant medications was documented. Assessment was made and recorded as to whether or not the use of the concomitant medication prohibited the volunteer to continue in the study or not.

5.6.2 Physical examination

A physical examination was performed on each volunteer at screening and again at the post-study follow-up examination or upon withdrawal. Changes from the physical examination at screening, were documented in the CRF.

5.6.3 Measurement of vital signs and body temperature

Vital signs, pulse, systolic and diastolic blood pressure were measured in the supine position (after 5 minutes rest) and standing after 3 minutes at screening, at each of the three admission periods and post study follow-up. Body temperature was measured at all visits at the same time as vital signs were measured.

5.6.4 Laboratory investigations

The clinical pathology laboratory, Drs Vermaak and Partners in Pretoria, South Africa performed all laboratory investigations (except urine analyses, drugs of abuse urine screen, the alcohol saliva test and the urine pregnancy test, which were performed at the research site).

Blood and urine were assayed as part of the screening procedures and were re-evaluated for post-study follow-up or upon withdrawal. The following variables were determined:

Haematology:	leucocytes, erythrocytes, haemoglobin, haematocrit, MCV, MCH, MCHC, platelets, neutrophils, eosinophils, lymphocytes, monocytes and basophils.
Serum biochemistry:	sodium, potassium, urea, creatinine, total protein, albumin, total bilirubin, conjugated bilirubin, ALP, GGT, AST, ALT and glucose (fasting at screening only).
Urine analysis:	Specific gravity, pH, protein, glucose, ketones, bilirubin, blood and urobilinogen (Bayer 10SG Multistix®).
Drugs of abuse screen:	Urine – 6 panel Medtox Diagnostics® (at screening visit only). This is a one-step immuno chromatographic test for the rapid, qualitative detection of one or more of the following: cannabinoids (THC), opiates, amphetamines, cocaine, benzodiazepines, methylenedioxymethyl amphetamine in human urine.
Alcohol saliva test:	Alco-Screen® (at screening visit only)
Urine pregnancy test:	Female volunteers (except those with a hysterectomy) (at screening, admission and post-study visit)

Results of pre-study laboratory investigations indicating values for one or more variables outside the normal ranges did not necessarily lead to exclusion of a volunteer from the study. They were interpreted in the clinical context. The investigator assessed the relevance of each laboratory value (at screening and post-study) outside the normal ranges. He or she wrote 'NCR' or 'CR' on the printed laboratory report next to each value outside the normal range, initialling each one. Any clinically relevant abnormal value were recorded in the CRFs. Post-study laboratory investigations indicating values outside the normal ranges could be repeated to establish if and when those values returned to within the normal ranges.

Values outside the normal ranges were, however, reviewed in the clinical context, and with regard to other relevant investigations and also in light of the specific IPs administered, before a decision was taken to repeat the investigation(s) in question. All clinically relevant

changes from screening that were observed post-study (including those where the post-study value remained within the normal range) were recorded as AEs in the CRFs by the investigator.

5.7. Statistical methods- Variables calculated

5.7.1 Baseline characteristics

Demographic data of individual volunteers were tabulated, together with summary statistics for each variable. Medical or surgical history, non-pharmacological therapy as well as physical examination abnormalities were presented for each volunteer.

5.7.2 Concomitant medication

The following information was presented for each volunteer who received any medication during the study: the study period during which the concomitant medication was taken, the investigational product administered at the start of the study period during which concomitant medication was taken, date of IP dosing, start date and time of administration, stop date and time of administration and indication.

5.7.3 Pharmacokinetic parameters and data

To compare the pharmacokinetics of meloxicam, paracetamol and codeine phosphate following single-dose administration in the fasted state, the following were calculated for each volunteer using the actual sampling intervals (relative to medication administration):

Plasma

- Area under the plasma concentration-time curve (AUC_{0-t})
- Maximum observed concentration (C_{max})
- Time to the maximum observed concentration (t_{max})
- Apparent terminal phase elimination half-life ($t_{1/2,z}$)
- Area under the plasma concentration-time curve to the time of the last quantifiable plasma concentration extrapolated to infinity ($AUC_{0-\infty}$)
- Mean residence time extrapolated to infinity ($MRT_{0-\infty}$).

5.7.4 Safety data

Data regarding pre- and post-dose laboratory investigations (clinical chemistry, haematology and urinalysis values), as well as adverse event data were collected for all volunteers. All haematological and serum biochemistry results from pre- and post-study investigations were summarised by descriptive statistics. The number of volunteers with laboratory values outside the "normal ranges" as well as individual data for all variables is included in the report. The incidence of adverse events was tabulated, and a discussion and detailed exposition of the adverse events presented in the final study report. Data for all vital signs has been tabulated for each volunteer and time point, together with descriptive statistics.

5.8. Results

5.8.1 Study volunteers

5.8.1.1 Disposition of volunteers

Of the 29 volunteers screened for the study, 24 were admitted to the research facility for all study periods (Appendix D). There was one volunteer who withdrew consent during treatment period 1. Twenty-three volunteers completed the study. The demographic data for the volunteers who took part in the study are summarised below in Table 5.1.

Table 5.1: Summary of volunteer demographics

	Age	Height	Weight
	(years)	(cm)	(kg)
N	24	24	24
Mean	22.5	176.0	71.48
Median	21.5	177.5	72.00
SD	3.74	9.94	12.270
Min	18	159	52.1
Max	37	194	92.3

5.8.1.2 Previous and current medical conditions

No clinically relevant surgical history and past or ongoing current medical conditions were recorded at screening.

5.8.1.3 Previous and concomitant medication

Four of the female volunteers were taking an oral contraceptive at screening. No other concomitant medication was taken at screening.

5.8.1.4 Physical examination at screening

No clinically relevant abnormalities were discovered during the physical examinations of the volunteers at screening.

5.8.1.5 Vital signs at screening

The vital sign measurements for all volunteers at screening were within the ranges specified in the entry criteria.

5.8.1.6 ECG at screening

No clinically significant abnormal ECGs were recorded at screening.

5.8.1.7 Laboratory assessments at screening

No screening laboratory values outside the normal ranges for haematology, serum biochemistry or urine analyses were reported as clinically relevant.

5.8.1.8 Social habits at screening

Eighteen volunteers (75 %) were non-smokers and 6 volunteers (25 %) were considered moderate smokers (less than 10 cigarettes/cigars/pipes per day). One volunteer (4 %) did not drink alcohol and the alcohol intake of the remaining 23 volunteers (96 %) was considered moderate (≤ 28 units per week).

5.8.2 Measurement of treatment compliance

All investigational products were administered by trained study personnel. Mouth checks were performed to confirm that the volunteers had swallowed the IP. The actual dosing times were recorded directly in the CRFs.

5.8.3 Calculation of pharmacokinetic results

Results were calculated using the Watson™¹ Laboratory Information Management System (LIMS). Peak areas were electronically imported directly into the Watson™ LIMS version 7.1 software from the Analyst version 1.4.1 software via the digital interface. Data were automatically summarised, calibration curves calculated according to pre-set regression equations and concentrations interpolated by the program. The Watson™ Laboratory Information Management System has been validated to 21 CFR Part 11 requirements by Farmovs-Parexel in collaboration with the Parexel Corporate Validation Committee.

5.8.4 Reporting of results

5.8.4.1 Plasma paracetamol concentrations

The results tabulated in this report are the final concentrations of paracetamol in plasma for all the samples after the collation of all the assay results, incorporating the repeated assay results. Plasma paracetamol concentrations for all volunteers for the test product are presented in Figure 5.1, reference product presented in Figure 5.2, mean plasma paracetamol concentrations (linear scale) versus scheduled sampling intervals for each product are presented in Figure 5.3 and mean plasma paracetamol concentrations (log scale) versus scheduled sampling intervals for each product are presented in Figure 5.4.

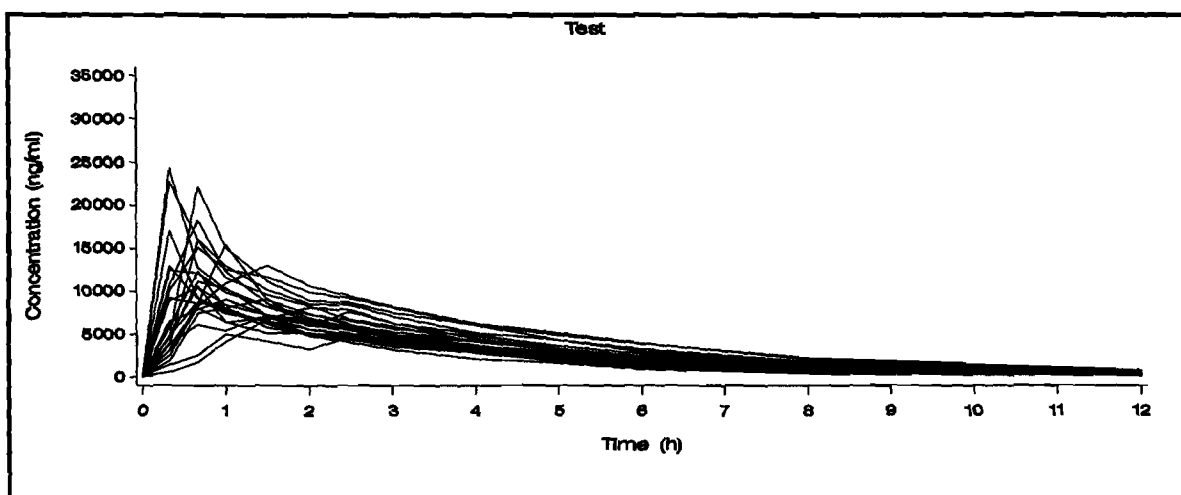


Figure 5.1: Plasma paracetamol concentrations for all trial volunteers – Test (Triple FDC)

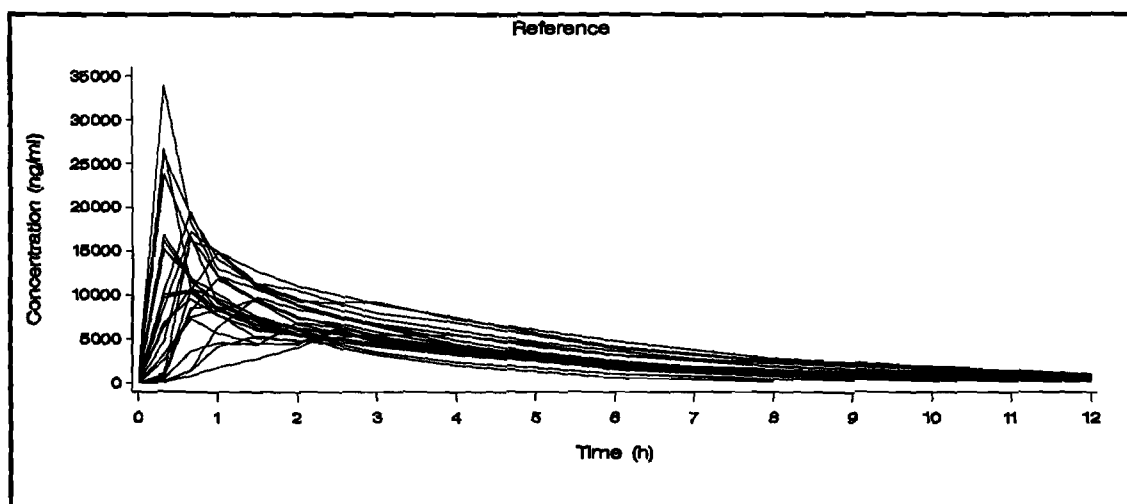


Figure 5.2: Plasma paracetamol concentrations for all trial volunteers – Reference (Myprodol®)

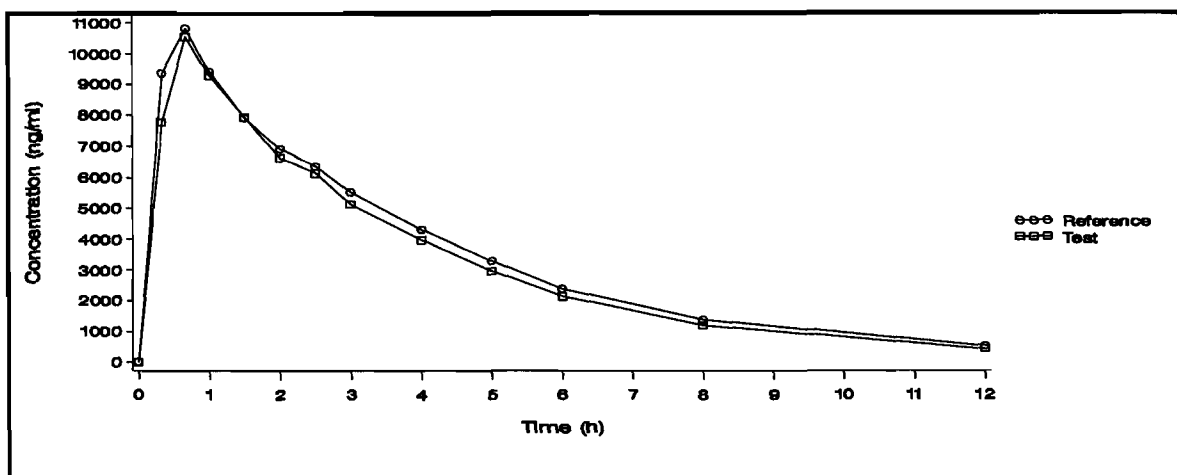


Figure 5.3: Mean plasma paracetamol concentrations (linear scale)

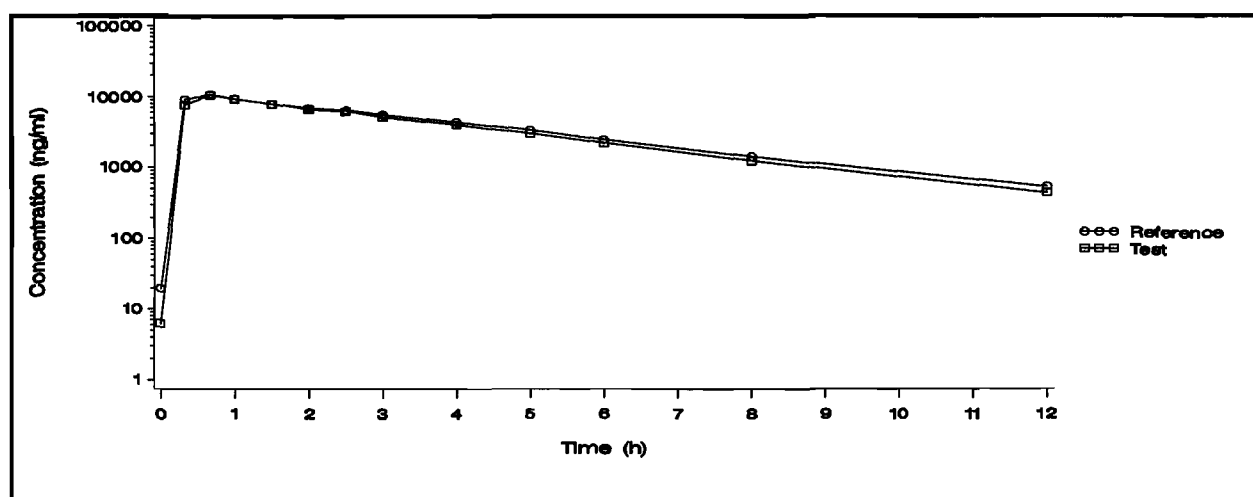


Figure 5.4: Mean plasma paracetamol concentrations (Log scale)

The mean, SD and 90 % conventional confidence intervals based on the paracetamol results from the ANOVA and the point estimates for the computed parameters are summarised in Table 5.2.

Table 5.2: Statistical analysis of pharmacokinetic parameters for paracetamol

Variable (unit)	Summary statistics	Test	Reference	Conventional 90 % CI	Point estimate*
AUC _{0-t} (h.ng/ml)	Mean SD	38982.838 11024.968	41935.052 13548.139	89.14 - 98.30 %	93.61 %
C _{max} (ng/ml)	Mean SD	12921.413 5206.413	14569.446 7256.230		

* Based on ln transformed data.

AUC_{0-t} analysis

A point estimate for the ratio of AUC_{0-t} of the test product relative to the reference product was 93.61 % with a 90 % t-confidence interval of 89.14 % - 98.30 %, Table 5.2. The intra-subject variability for AUC_{0-t} was 9.62 %.

C_{max} analysis

A point estimate for the ratio of C_{max} of the test product relative to the reference product was 92.97 % with a 90 % t-confidence interval of 80.32 % - 107.62 %, Table 5.2. The intra-subject variability for C_{max} was 29.31 %. Summary statistics of the secondary parameters for the paracetamol results are presented in Table 5.3.

AUC_{0-∞} analysis

The extrapolation of AUC_{0-∞} was not more than 20 % in any volunteer.

Table 5.3: Statistics (untransformed) of secondary pharmacokinetic parameters for paracetamol

Variable (unit)	Summary statistics	Test	Reference
AUC _{0-∞} (h.ng/ml)	Mean	40680.060	43957.042
	SD	11717.881	14398.765
MRT _{0-∞} (h)	Mean	3.793	3.934
	SD	0.550	0.842
t _{1/2,z} (h)	Mean	2.514	2.540
	SD	0.504	0.467
t _{max} (h)	Median	0.667	0.667

5.8.4.2 Plasma codeine concentrations

The results tabulated in this report are the final concentrations of codeine in plasma for all the samples after the collation of all the assay results, incorporating the repeated assay results. Plasma codeine concentrations for all volunteers for the test product are presented in Figure 5.5, reference product are presented in Figure 5.6, mean plasma codeine concentrations (linear scale) versus scheduled sampling intervals for each product are presented in Figure 5.7 and mean plasma codeine concentrations (log scale) versus scheduled sampling intervals for each product are presented in Figure 5.8.

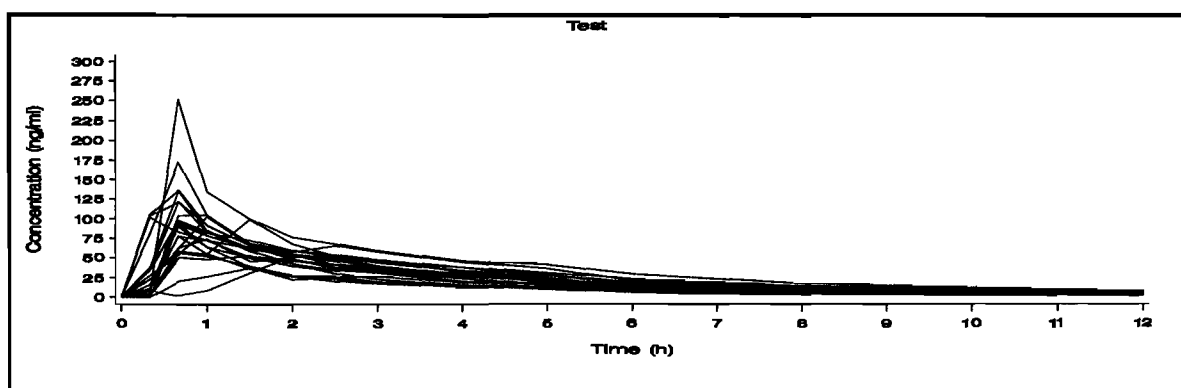


Figure 5.5: Plasma codeine concentrations for all trial volunteers – Test (Triple FDC)

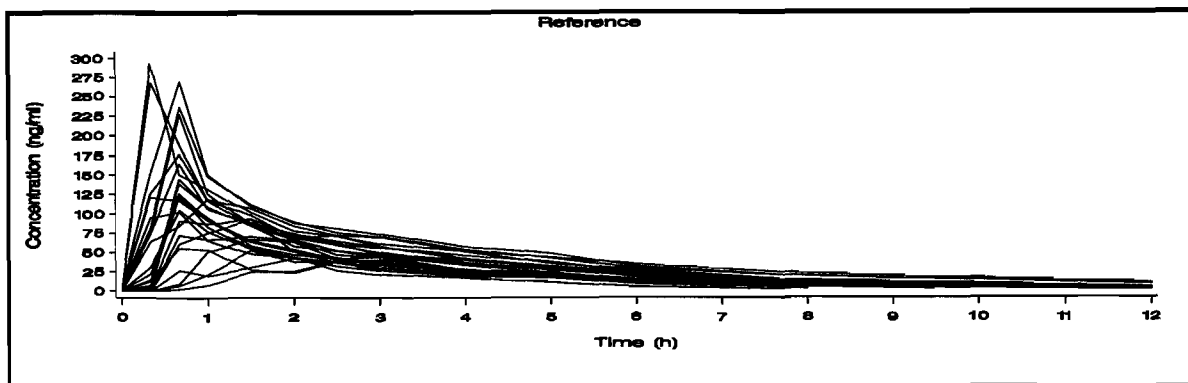


Figure 5.6: Plasma codeine concentrations for all trial volunteers – Reference (Myprodol®)

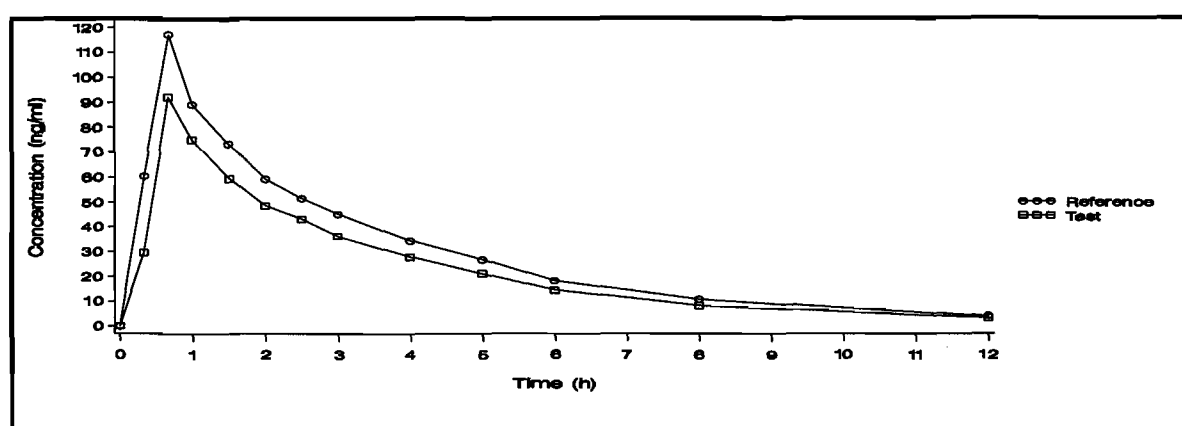


Figure 5.7: Mean plasma codeine concentrations (linear scale)

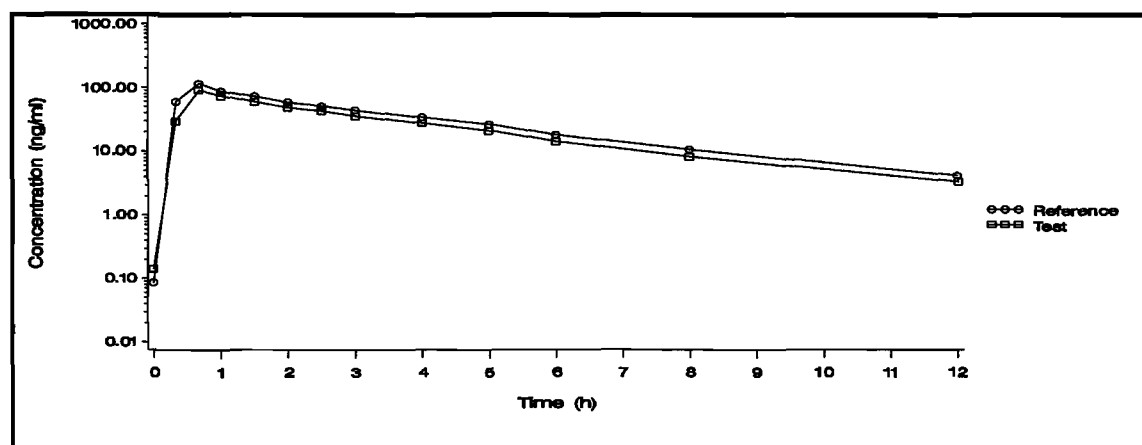


Figure 5.8: Mean plasma codeine concentrations (Log scale)

The mean, SD and 90 % conventional confidence intervals based on the codeine results from the ANOVA and the point estimates for the computed parameters are summarised in Table 5.4.

Table 5.4: Summary of statistical analysis of pharmacokinetic parameters for codeine

Variable (unit)	Summary statistics	Test	Reference	Conventional 90 % CI	Point estimate*
AUC _{0-t} (h.ng/ml)	Mean	276.229	351.484	74.14 % - 87.35 %	80.47 %
	SD	90.929	141.109		
C _{max} (ng/ml)	Mean	101.713	135.975	66.77 % - 96.25 %	80.17 %
	SD	44.847	74.568		

*Based on ln transformed data.

AUC_{0-t} analysis

A point estimate for the ratio of AUC_{0-t} of the test product relative to the reference product was 80.47 % with a 90 % t-confidence interval of 74.14 % - 87.35 %, Table 5.4. The intra-subject variability for AUC_{0-t} was 16.19 %.

C_{max} analysis

A point estimate for the ratio of C_{max} of the test product relative to the reference product was 80.17% with a 90 % t-confidence interval of 66.77 % - 96.25 %, Table 5.4. The intra-subject variability for C_{max} was 37.06 %. Summary statistics of the secondary parameters for the codeine results are presented in Table 5.5.

AUC_{0-∞} analysis

The extrapolation of AUC_{0-∞} was not more than 20 % in any volunteer.

Table 5.5: Summary statistics (untransformed) of secondary pharmacokinetic parameters for codeine

Variable (unit)	Summary statistics	Test	Reference
AUC _{0-∞} (h.ng/ml)	Mean	289.838	368.603
	SD	96.598	151.359
MRT _{0-∞} (h)	Mean	3.779	3.838
	SD	0.510	0.662
t _{1/2,z} (h)	Mean	2.596	2.681
	SD	0.498	0.465
t _{max} (h)	Median	0.667	0.667

5.8.4.3 Plasma meloxicam concentrations

The results tabulated in this report are the final concentrations of codeine in plasma for all the samples after the collation of all the assay results, incorporating the repeated assay results. Plasma meloxicam concentrations for all volunteers for the test product are presented in Figure 5.9, reference product are presented in Figure 5.10, mean plasma meloxicam concentrations (linear scale) versus scheduled sampling intervals for each product are presented in Figure 5.11 and mean plasma meloxicam concentrations (log scale) versus scheduled sampling intervals for each product are presented in Figure 5.12.

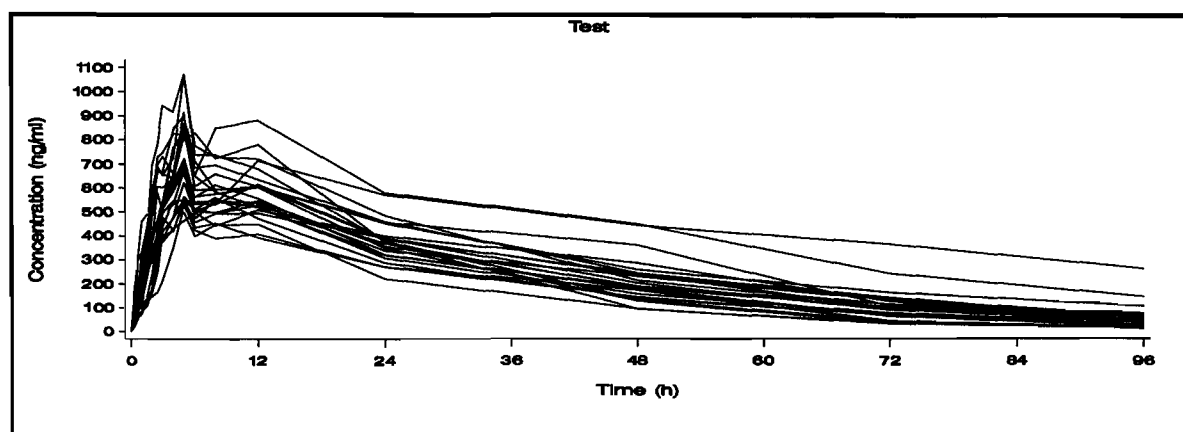


Figure 5.9: Plasma meloxicam concentrations for all trial volunteers – Test (Triple FDC)

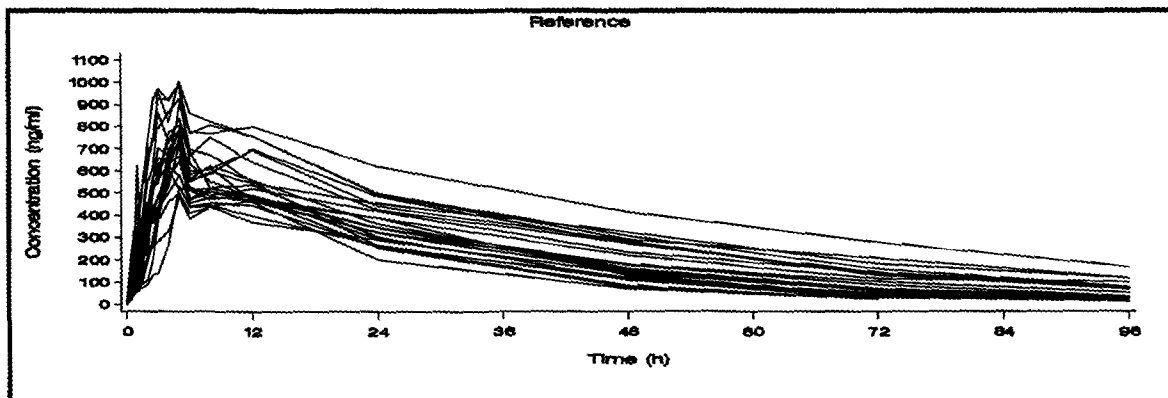


Figure 5.10: Plasma meloxicam concentrations for all trial volunteers – Reference (Mobicl®)

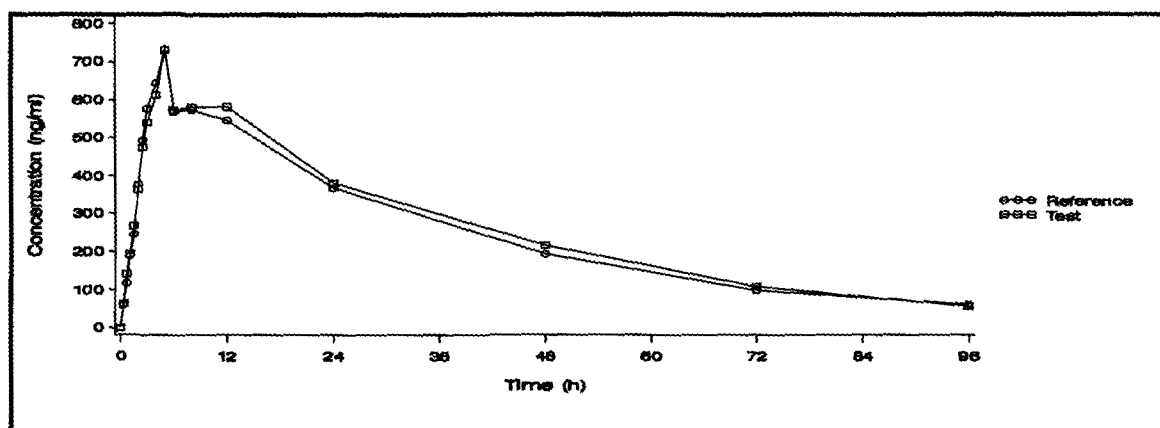


Figure 5.11: Mean plasma meloxicam concentrations (linear scale)

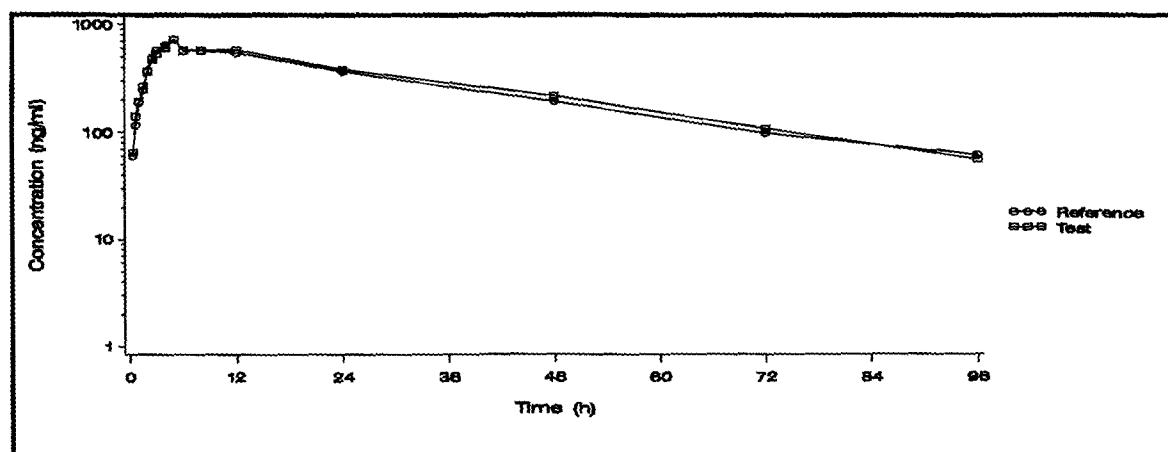


Figure 5.12: Mean plasma meloxicam concentrations (log scale)

The mean, SD and 90% conventional confidence intervals based on the meloxicam results from the ANOVA and the point estimates for the computed parameters are summarised in Table 5.6.

Table 5.6: Statistical analysis of pharmacokinetic parameters for meloxicam

Variable (unit)	Summary statistics	Test	Reference	Conventional 90 % CI	Point estimate*
AUC _{0-t} (h.ng/ml)	Mean	25011.089	23641.454	99.77 - 114.17%	106.73%
	SD	7042.012	7642.946		
C _{max} (ng/ml)	Mean	734.226	738.626	93.31 - 104.58%	98.79%
	SD	168.631	155.338		

*Based on ln transformed data.

AUC_{0-t} analysis

A point estimate for the ratio of AUC_{0-t} of the test product relative to the reference product was 106.73 % with a 90 % t-confidence interval of 99.77 % - 114.17 %, Table 5.6. The intra-subject variability for AUC_{0-t} was 13.29 %.

C_{max} analysis

A point estimate for the ratio of C_{max} of the test product relative to the reference product was 98.79 % with a 90 % t-confidence interval of 93.31 % - 104.58 %, Table 5.6. The intra-subject variability for C_{max} was 11.22 %.

Summary statistics of the secondary parameters for the meloxicam results are presented in Table 5.7 below.

AUC_{0-∞} analysis

The extrapolation of AUC_{0-∞} was more than 20 % in one volunteer using the test product.

Table 5.7: Statistics (untransformed) of secondary pharmacokinetic parameters for meloxicam

Variable (unit)	Summary statistics	Test	Reference
AUC _{0-∞} (h.ng/ml)	Mean	27760.652	25951.824
	SD	11159.418	9853.604
MRT _{0-∞} (h)	Mean	37.350	35.410
	SD	14.804	11.097
t _{1/2,z} (h)	Mean	24.231	23.654
	SD	10.825	7.688
t _{max} (h)	Median	5.000	5.000

5.8.5 Pharmacokinetic conclusions

Paracetamol and meloxicam, as present in the Triple FDC capsules, were comparable with the reference products in respect of AUC_{0-t} and C_{max}. However, codeine was less bioavailable from the Triple FDC capsule than from Myprodol®. The reason for this observation is uncertain but it correlates to the decreased stability results of codeine phosphate in the Triple FDC capsule in Chapter 4. The study design does not permit meaningful interference in this regard.

5.9 Safety evaluation

5.9.1 Analysis of Safety data

All haematological and serum biochemistry results from pre- and post-study investigations (n = 24) are summarised by descriptive statistics. The number of volunteers with laboratory investigations outside the reference ranges is reported, as well as individual data for all variables. Adverse events are listed on an individual basis.

5.9.2 Brief summary of adverse events

All adverse events are listed per volunteer in Table 5.8. The investigational products were well-tolerated. AEs that were recorded were all reported spontaneously by the volunteers, elicited through non-directed interviews or observed by the investigators throughout the study. No serious adverse events were reported during this study and none of the

volunteers withdrew or was withdrawn from the study due to an adverse event. No adverse events related to laboratory variables were reported.

Table 5.8: Summary of adverse events

Product administered at the onset of the AE	MedDRA System organ class / Preferred term	Number of AEs
Triple FDC Capsule	Eye disorders: Keratitis	1
	Gastrointestinal disorders: Enteritis	1
	Infections and infestations: Gastroenteritis	1
Mobic®	Nervous system disorders: Headache	1
Myprodol®	Gastrointestinal disorders:	1
	Nausea	

5.9.2.1 Analysis of adverse events

A total of 5 adverse events were reported by 5 of the 24 volunteers. Of these, 1 adverse event was assessed as not related to the study medication, and all others to be improbably related to the study medication. All adverse events were of mild intensity, except for 1 that was of moderate intensity.

Gastrointestinal disorders/infections and infestations were the predominant groups of adverse events reported. One volunteer reported enteritis during the third admission period, 76.6 hours after receiving the test product. One volunteer reported gastroenteritis during the third admission period, 72.8 hours after receiving the test product. One volunteer reported nausea during the third admission period, 72.7 hours after receiving the reference product, Myprodol®. This event was considered to be improbably related to the investigational product and to be of mild intensity.

Apart from gastrointestinal disorders/infections and infestations, 1 headache and 1 occurrence of keratitis was reported. One volunteer reported keratitis during the third admission period, 66.4 hours after receiving the test product. This event was considered not related to the investigational product and to be of moderate intensity. One volunteer reported a headache during the third admission period, 80.7 hours after receiving the reference product, Mobic®. The investigator considered this event improbably related to the investigational product and to be of mild intensity. In assessing the relationship of each adverse event to the study medication, the investigator took into account the time of administration of study medication and the frequency of the occurrence of a particular adverse event over the study period.

5.9.3 Deaths and other serious adverse events

There were no deaths or other serious adverse events reported during the study.

5.9.4 Concomitant medication

To our knowledge, all subjects, other than one, refrained from using any medication, including those sold over the counter, for 7 days before the first administration of study medication and for the duration of the study, with the exception of the study medication taken on clinic days/during treatment phases, and of hormonal contraceptives, where applicable. The volunteer used 2 doses of 0.3 ml/0.3 mg diclofenac eyedrops twice, as well as 600 mg aspirin once, starting approximately 3 days after administration of the test product during the third admission period for the treatment of UV keratitis.

5.9.5 Clinical laboratory evaluation

None of the laboratory results were outside the normal reference ranges.

5.9.5.1 Evaluation of each laboratory parameter

Laboratory values over time

No clinically significant abnormalities in the post-study safety laboratory parameters were reported.

Individual volunteer changes

No clinically significant changes were observed when comparing the safety laboratory parameters obtained at screening and at the post-study follow-up examinations.

Individual clinically significant abnormalities

No clinically significant abnormalities for any of the screening and post-study safety laboratory parameters were reported.

5.9.6 Safety and tolerability conclusion

In general, the investigational products were well tolerated by healthy volunteers in this study. No deaths or serious adverse events were reported during the study and no adverse event was of severe intensity.

Electrocardiogram evaluations performed at screening only, were compatible with expectations for a healthy male and female population.

There was no clinically significant and/or consistent drug-related change in vital signs, physical findings or safety laboratory values after administration of the investigational products.

No pathological findings were observed during the post-study physical examination and laboratory tests.

5.9.6.1 Post-study follow-up

The post-study safety evaluation was done after collection of the 96-hour post-dose sample of the third study period or, in the case of a drop-out, within 72 hours of withdrawal from the study.

5.10 Discussion

5.10.1 Study population

A total of 29 volunteers were screened of whom 24 were randomised. There was one volunteer who withdrew consent during treatment period 1. Twenty-three volunteers completed the study.

5.10.2 Safety

In general, the investigational products were well tolerated by healthy volunteers in this study. There was no clinically significant and/or consistent drug-related change in vital signs, physical findings or safety laboratory values after administration of the investigational products.

5.10.3 Concomitant medication

Drospirenone, ethinylestradiol, diclofenac and aspirin were administered by some of the subjects during this study. The very high specificity of the LC-MS/MS assay procedure precludes the detection of any compounds that do not possess the capability to produce the specific precursor ion followed by formation of the specific product ion produced and monitored in the mass spectrometer. Interference from the concomitant medication was neither expected nor observed during the assay of the study samples.

5.11 Conclusion

The results of the analyses describe the different pharmacokinetic variables for the three components of the Triple FDC capsule in respect to the reference products. The purpose of the study was not to establish bioequivalence but rather to determine relative bioavailability of the active ingredients of the fixed dose combination capsules in respect of the reference products. Paracetamol and meloxicam, as present in the Triple FDC capsules, were comparable with the reference products in respect of AUC_{0-t} and C_{max} . However, codeine was less bioavailable from the Triple FDC capsules when compared to Myprodol®. The reason for this observation is uncertain but correlates to the assay and dissolution results of the product during stability testing. The stability results and further investigations carried out are detailed in Chapter 4.

CHAPTER 6

PROOF OF EFFICACY AND SAFETY STUDY

INTRODUCTION

In this study the Triple fixed-dose combination (FDC) product, intended for pain relief, was evaluated for analgesic efficacy and safety in the treatment of dysmenorrhoea. It is postulated that a combination of meloxicam, an NSAID of the oxicam class, with paracetamol and codeine phosphate will act synergistically in providing relief of pain associated with dysmenorrhoea. This postulate is supported by the rat study findings on the synergistic analgesic activity shown in Chapter 3. According to Mehlich & Fulmer (1997:83), dysmenorrhoea is an established model for assessing pain relieving qualities of analgesic candidates. This view was supported by Zhang & Li Wan Po (1998:780-789). The Committee for Proprietary Medicinal Products (2003) lists dysmenorrhoea as an appropriate model for therapeutic confirmatory studies with regard to mild to moderate acute pain (Raffa, 2001:257).

6.1 Dysmenorrhoea

Dawood (1988:23) reported that dysmenorrhoea affects at least 50 % of reproductive-aged women, and in approximately 10 % of women it is severe enough to interfere with their daily activity. Affected individuals experience a range of associated symptoms including nausea, dizziness, headache and backache.

Primary dysmenorrhoea is characterised clinically as painful cramping in the lower abdomen occurring just before or during menstruation in the absence of pelvic pathology, mediated mainly by excessive secretion of prostaglandins during the late luteal phase of the menstrual cycle (Budolf, 1987:453-464; Coco, 1999:489-496). Mehlich & Fulmer (1997:83-92) explained that dysmenorrhoea may be "primary" (or idiopathic), which occurs during ovulatory cycles, or "secondary" to some type of pelvic pathology (e.g. intrauterine devices, endometriosis, pelvic inflammatory disease, ovarian cyst, etc). Symptoms of dysmenorrhoea are similar to pelvic inflammatory disease and ectopic pregnancy, which are serious and life threatening; thus, a physician should rule out the presence of secondary causes of dysmenorrhoea.

Many women use non-prescription (over-the-counter) products to treat abdominal pain and cramping, headache, backache and other symptoms. Nonsteroidal anti-inflammatory drugs (NSAIDs) are effective in treating dysmenorrhoea in 80 to 85 % of women. Ideally, NSAIDs therapy should be started with the earliest onset of pain. To be most effective, NSAIDs should be started during the last week of the luteal phase before the onset of dysmenorrhoea and continued through menstruation on a schedule instead of an as-needed basis (Dawood, 1988:23-29). However, to standardise clinical research study conditions, it is necessary to allow initial pain to reach a moderate to severe intensity before the first dose of an analgesic is taken (Daniels *et al.*, 2002:350 & Bitner *et al.*, 2004:340-345). Due to the inter-ethnic differences in the pharmacokinetics of meloxicam and codeine phosphate explained in Chapter 5, only caucasians females were enrolled in the study.

6.2 Study title

A multiple dose, double-blind, double-dummy, randomised, placebo- and active-controlled cross-over, proof-of-concept study was conducted to determine the safety and efficacy of two different doses of the Triple FDC capsule (10 mg codeine phosphate, 250 mg paracetamol and 2.5 mg meloxicam) versus a Double FDC capsule (250 mg paracetamol and 10 mg codeine phosphate) or placebo in females with primary dysmennorhoea, a Phase II study.

The study was conducted at a single center QdotPharma, a Division of Parexel International (South Africa) (Pty) Ltd.

6.3 Study protocol

6.3.1 Independent Ethics Committee (IEC)

The study protocol, volunteer diaries, volunteer information leaflet, volunteer information and consent form were reviewed and approved by the Pharma-Ethics Independent Research Ethics Committee. The South African Medicines Control Council (MCC) reviewed and approved the protocol, and authorised the use of the Investigational products (IPs) for the duration of the trial as per protocol.

6.3.2 Ethical conduct of the study

This study was conducted and informed consent obtained, according to the ethical principles stated in the Declaration of Helsinki (52nd WMA General Assembly) and the ICH Guideline for Good Clinical Practice (1997) (GCP).

The conduct of the study complied with the following recommendations and guidelines:

- Guidelines for good practice in the conduct of clinical trials in human participants in South Africa: Clinical Trials Guidelines. SA Department of Health. 2000
- The Declaration of Helsinki (52nd WMA General Assembly)
- The ICH Guideline for Good Clinical Practice (1997).

Qdot Pharma obtained a written approval of the study protocol from the Pharma-Ethics Independent Research Ethics Committee and the MCC before initiation of screening procedures.

6.3.3 Volunteer information and Informed consent

Before commencement of the study, the volunteer information leaflet and consent form, as approved by the IEC, were provided to each volunteer. The investigators explained both verbally and in writing, to all volunteers the nature, purpose and risks of the screening procedures, as well as the nature, purpose, obligations, restrictions and risks relevant to the study. Volunteers were informed that they were not in any way obliged to participate in the study, and they were given ample opportunity to enquire about the study detail. It was made clear that refusal to participate or withdrawal from the study at any stage would not prejudice a volunteer's subsequent care.

6.4 Study objectives

The study objectives were divided into two, primary and secondary objectives.

6.4.1 Primary objectives

6.4.1.1 To determine the summed pain intensity difference for 0 to 8 hours post first dose of study medication (SPID₀₋₈) of two different doses of a Triple FDC capsule compared to a Double FDC capsule or Placebo.

6.4.1.2 To determine the safety and tolerability of two different doses of the Triple FDC capsules compared to a Double FDC capsule or placebo over a 72 hour treatment period.

6.4.2 Secondary objectives

- To determine total pain relief 0 to 8 hours (TOTPAR₀₋₈), 0 to 12 hours (TOTPAR₀₋₁₂) and 0 to 24 hours (TOTPAR₀₋₂₄) post first dose of study medication of two different doses of the Triple FDC capsules compared to a Double FDC capsules or placebo.
- To evaluate the intensity of pain at baseline (0 hours), 30, 60 and 90 minutes and at 2, 3, 4, 5, 6, 7, 8, 12 and 24 hours (PID).
- To determine the summed pain intensity difference 0 to 12 hours (SPID₀₋₁₂) and 0 to 24 hours (SPID₀₋₂₄) after the first dose of IP of two different doses of the Triple FDC capsules compared to the Double FDC capsules or placebo.
- To determine the percentage of volunteers who require rescue medication during each treatment period with Triple FDC capsules as compared to placebo.
- To determine the time to the first administration of rescue medication with two different doses of Triple FDC capsules compared to a Double FDC capsules or placebo.
- To determine the percentage of IP used per treatment period.
- To obtain a global evaluation of the IP at 72 hours or early termination.

6.5 Investigational plan

6.5.1 Overall study design and plan

The study was conducted according to a multiple-dose, double-blind, double-dummy, active and placebo-controlled, randomised, two-part crossover design to determine the safety and efficacy of two different doses of a Triple FDC capsules compared to a Double FDC capsules or matching placebo, administered three times daily in 24 hours and thereafter as needed for 3 days, in otherwise healthy females with primary dysmenorrhoea. Before administering the first dose of study medication during the next consecutive menstrual cycle when they experience moderate to severe menstrual cramping pain, volunteers were required to perform a pregnancy test.

Volunteers self-medicated during four evaluable menstrual cycles, once with each study medication. Volunteers were allowed to miss one menstrual cycle at any stage of the trial when menstrual cramping pain was not of a moderate to severe nature. In this instance, no investigational product (IP) was taken; the volunteer was required to attend the research clinic for an unscheduled visit where the IP and study materials were replaced. Treatment was then held over to the next consecutive menses.

Table 6.1 outlines the scheduled study procedures carried out at various visit during the study. Before leaving the research site, each volunteer was given an identification card with the instructions that they must keep it with them for the duration of the study and that, should the need arise to consult another clinician, they should produce the card.

6.5.1.1 Cycle 1: Visit 1- screening

The volunteers reported to the clinic 18 to 21 days before the expected date of the next menses. They were assessed according to the inclusion and exclusion criteria for participation in the study.

6.5.1.2 Cycle 1: Visit 2 - Treatment period 1

Volunteers reported to the clinic 7 ± 2 days after visit 1, to confirm their eligibility to continue in the study. They were questioned regarding adverse events (AEs) experienced since visit 1 and medications taken in that period. Eligible volunteers were randomised to receive the IPs according to the randomisation schedule.

6.5.1.3 Cycle 2: Visit 3 - Treatment period 2

Volunteers reported to the clinic within 8 days including day 8, after first dose of study medication in treatment period 1 to review the data recorded in their diaries and to confirm their eligibility to continue in the study. Volunteers were questioned regarding adverse events and concomitant medications.

6.5.1.4 Cycle 3: Visit 4 - Treatment period 3

Volunteers reported back to the clinic within 8 days including day 8, after the first dose of IPs in treatment period 2 to review the data recorded in the study diaries and to confirm their eligibility to continue in the study. Volunteers were questioned regarding adverse events and concomitant medications.

6.5.1.5 Cycle 4: Visit 5 - Treatment period 4

Volunteers reported back to the clinic within 8 days including day 8, after the first dose of IPs in treatment period 3 to review the data recorded in the study diaries and to confirm their eligibility to continue in the study. Volunteers were questioned regarding adverse events and concomitant medications.

6.5.1.6 Cycle 4: Visit 6 - Post-study follow-up

The post-study safety evaluation were done within 8 days including day 8, after the ingestion of the first dose of study medication in treatment period 4 or, in the case of a drop-out, within 72 hours of withdrawal from the study.

6.5.1.7 Unscheduled visit

Volunteers reported back to the clinic within 8 days including day 8, after the first day of the untreated menstrual period to review the data recorded in the study diaries and to confirm their eligibility to continue in the study. Volunteers were questioned regarding adverse events and concomitant medications.

Volunteers were instructed to return to the research site within 8 days including day 8, after taking the first dose of study medication to review the treatment cycle with study staff and for final study procedures.

6.5.2Diaries

Volunteers will be required to complete a diary for each study period (Appendix E). The following items are to be recorded in the diary:

- pain intensity

- pain relief
- global evaluation of the drug's performance
- details of time of administration of study medication (or use of rescue medication if appropriate)
- time of performance and results of the urine pregnancy test prior to ingestion of study medication
- adverse events and concomitant medications

6.5.3 Pain recording and rating scales

Pain was evaluated by the volunteers at specified times and recorded in the study diaries. Volunteers who took the IP late in the day or at night will be required to complete all specified efficacy evaluations described below, as meticulously as possible.

6.5.3.1 Pain intensity

Pain intensity will be recorded at baseline (0 hours), 30, 60 and 90 minutes and at 2, 3, 4, 5, 6, 7, 8, 12 and 24 hours after the first dose of the IP for each menstrual cycle including a cycle for which IP is not required (if applicable).

In order to qualify for treatment with IP, pain associated with menstruation must reach the intensity of moderate to severe. The pain intensity was rated according to the rating described in paragraph 6.5.11.2.

6.5.3.2 Pain relief

Times for recordings: 0 hours (at baseline, immediately before taking the first dose of study medication), 30, 60 and 90 min and at 2, 3, 4, 5, 6, 7, 8, 12 and 24 hours after the first dose of IP. The time each dose is taken must be recorded according to the 24-hour clock.

Total pain relief will be recorded at the above times according to a 5-point rating described in paragraph 6.5.11.2.

Table 6.1: Study Design for the screening examinations and investigations

Study Procedures	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Unscheduled	Post study ⁸
	Screening	Study Period				
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
Physical Examination	✓	✓ ³	✓ ³	✓ ³	✓ ³	✓
Diagnosis of primary dysmenorrhoea	✓					
Vital signs and body temperature	✓	✓	✓	✓	✓	✓
12-lead ECG	✓					
Haematology, Serum biochemistry	✓					✓
Urine analysis	✓	✓				✓
Urine drugs of abuse	✓	✓				
Alcohol saliva test	✓					
βhCG	✓ ¹	✓ ⁴	✓ ⁴	✓ ⁴	✓ ⁴	✓ ¹
Eligibility check		✓	✓	✓	✓	
Dispense IPs ² and pregnancy kits		✓	✓	✓	✓	
Retrieve unused IPs ²			✓	✓	✓	✓
Issue diary		✓	✓	✓	✓	
Collect and review diary			✓	✓	✓	✓
Pain relief recording		✓ ^{5,6}	✓ ^{5,6}	✓ ^{5,6}	✓ ^{5,6}	
Pain intensity recording		✓ ⁷	✓ ⁷	✓ ⁷	✓ ⁷	
Con Meds and study restrictions check	✓	✓	✓	✓	✓	✓
AE reporting		Throughout the study				✓

1 Serum βhCG at screening and post study follow-up

2 including rescue medication

- 3 Brief physical check
- 4 Urine β hCG before first dose of IP
- 5 Pain relief to be recorded at 0 (immediately before first dose of study medication), 30, 60, 90 minutes and 2, 3, 4, 5, 6, 7, 8, 12, and 24 hours post-dose.
- 6 Analgesic action of drug to be rated at 72 hours post first dose of study medication
- 7 Pain intensity recording at baseline (0 hours), 30, 60, 90 minutes and 2, 3, 4, 5, 6, 7, 8, 12 and 24 hours. To be recorded for all menses if 4 consecutive menses are not treated with IP
- 8 Or within 72 hours of withdrawal

6.5.3.3 Global analgesic rating

Global analgesic rating was recorded at 72 hours after the first dose of the IP (or at the time of ingestion of the first dose of rescue medication) using the rating described in paragraph 6.5.11.2.

6.5.4 **Selection of study population**

The criteria were set to ensure a homogeneous study population that will facilitate the conduct and scientific evaluation of the study. The specific criteria have also been selected to minimise risk to the well-being of volunteers.

6.5.4.1 Inclusion criteria

- The volunteer was informed and was given ample time and opportunity to think about her participation and she was willing and able to comprehend and comply with all trial requirements. She gave written informed consent to participate in the study and to abide by study restrictions.
- The volunteer was an otherwise healthy, nulliparous or multiparous, Caucasian female, aged ≥ 18 years.
- The volunteer had a diagnosis of primary dysmenorrhoea.
- The volunteer experienced moderate to severe menstrual (abdominal) cramping pain consistently for at least 4 menstrual periods prior to entry into the study.
- The volunteer had regular menses every 22 to 33 days.
- The volunteer had no history of pelvic pathology.
- A gynaecological examination performed within 1 year prior to randomisation into the study, found no evidence of other causes of dysmenorrhoea.

- The volunteer was in general good health (apart from the study condition), as determined by a medical history, physical examination, 12-lead ECG, blood pressure, pulse rate, oral temperature and clinical laboratory results within the “normal” ranges for the relevant tests (unless the clinical investigator considered the deviation to be irrelevant for the purpose of the study).

6.5.4.2 Exclusion criteria

Female volunteers were excluded from the study for any of the following reasons, Volunteers who:

- had evidence or history of clinically relevant psychiatric, hepatic, renal, respiratory, cardiovascular, gastrointestinal, endocrine or central nervous system disease as determined at the screening visit.
- had a major illness during the 3 months before commencement of the study-related procedures.
- had a known clinically relevant gynaecological abnormality.
- were pregnant (positive serum β -hCG test at screening).
- were breastfeeding.
- had a history of peptic ulcer disease.
- had a history of bronchial asthma.
- were hypertensive with a resting (after 5 minutes) supine diastolic blood pressure greater than 90 mmHg, or a supine systolic blood pressure greater than 140 mmHg.
- had a pulse rate greater than 110 beats per minute or less than 50 beats per minute.
- had clinically relevant out-of-range values for haematology, serum chemistry or urinalysis parameters (ALT, AST and GGT values more than twice the upper limit of the normal range are regarded as clinically significant) as interpreted by the investigator.
- had a history of hypersensitivity to any drugs.
- had a history of any severe allergies.

participated in another study with any IP within 8 weeks before the first administration of the current IPs, or until at least 5 x $t_{1/2}$ elimination has lapsed, whichever was the greater.

- received treatment within the previous 3 months with any drug having a well-defined potential for adversely affecting a major organ or body system at the recommended doses.
- received over-the-counter medication, or a regular course of prescribed medication, during the 7 days before the start of the study.
- were taking any concomitant medication except contraceptives.
- had a history of, or current misuse of any substance of abuse.
- Positive urine screen for drugs of abuse at the screening visit.
- had a history of compulsive alcohol abuse (> 28 units per week, one unit is equivalent to 8 – 10 g of ethanol).
- were not able to comprehend and/or execute the protocol requirements e.g. keeping of a diary and compliance with medication.
- in the opinion of the Investigator, should not participate in the study.

6.5.5 Specific restrictions or requirements on volunteers

Volunteers were instructed to refrain from using any prescribed or over-the-counter medication for the duration of the study unless such medication was approved by the investigator. If any medicines, other than the prescribed IP, were required or indicated and whenever practical, the investigator was contacted immediately to discuss the most appropriate action before any medicines were taken. A decision to continue the volunteer's participation in the study was made based on time-dependent pharmacodynamic and pharmacokinetic properties of such medicines relative to the time the medication was administered and the possible influence of the ingested medication on the pharmacokinetics of the IP, interference with the assay method and well-being of the volunteer.

6.5.6 Treatments administered

Volunteers were assigned to a treatment order according to the randomisation schedule provided. They took prescribed treatments over four evaluable menses characterised by moderate to severe dysmenorrhoea. Volunteers were instructed to take 2 capsules (one

from each container), every 8 hours for the first 24 hours with a maximum of 3 doses in 24 hours, and thereafter as needed for a maximum of 3 days from the onset of menstrual pain.

6.5.6.1 Treatment A (Test product)

Triple FDC capsules containing 10 mg codeine phosphate, 250 mg paracetamol, 2.5 mg meloxicam per capsule for oral administration.

Dose: Two Triple FDC capsules (20 Triple FDC capsules).

6.5.6.2 Treatment B (Test product)

Triple FDC capsule containing 10 mg codeine phosphate, 250 mg paracetamol, 2.5 mg meloxicam per capsule for oral administration and a matching Placebo capsule.

Dose: One Triple FDC capsule and one matching Placebo capsule (10 Triple FDC capsules and 10 Placebo capsules)

6.5.6.3 Treatment C (Reference product)

Double FDC capsules containing 10 mg codeine phosphate and 250 mg paracetamol per capsule for oral administration

Dose: Two Double FDC capsules (20 Double FDC capsules)

6.5.6.4 Treatment D (Control product)

Placebo capsules to match test product for oral administration.

Dose: Two Placebo capsules (20 Placebo capsules)

6.5.6.5 Rescue medication

Paracetamol 500 mg tablets (Panado®)

Dose: One tablet four hourly as needed, with a maximum of 6 tablets in 24 hours, but not before one hour after taking study medication.

If rescue medication was taken, no further study medication was allowed to be taken during that particular menstrual cycle. The time of taking the rescue medication was documented in the diary as well as pain intensity and pain relief during the treatment period.

6.5.6.6 Storage and dispensing

The IPs were stored in a limited access area where the temperature did not exceed 25 °C. Before dispensing the IPs to each volunteer, the registered nurse/investigator/pharmacist checked each medication container and label, and also ensured that the volunteer randomisation number on the label corresponded to the number allocated to the appropriate volunteer. The volunteer's initials were documented on the medication label at the time of dispensing.

6.5.6.7 Drug inventory

The IPs were not used for purposes other than as directed by the protocol. The volunteer was required to record the number of capsules and Panado® tablets (paracetamol) taken during each treatment period, in their study diaries, as well as any other medication taken during the trial period. All unused medication and empty containers were returned to Qdot Pharma at visits 3, 4, 5, 6 and if required at an unscheduled visit.

6.5.7 Method of assigning volunteers to treatment groups

The randomisation schedule was generated electronically using the PROC PLAN procedure in SAS®. The randomisation schedule was generated in such a way to ensure the ratio between test products, reference product and placebo (1:1:1:1) is preserved within each treatment period.

6.5.8 Packaging

The packed IPs included both primary and secondary packaging. Primary packaging consisted of two securitainers, each containing 10 capsules (9 capsules for dosing and 1 capsule as back-up) as well as a securitainer for rescue medication containing 20 tablets (18 tablets as needed as rescue medication and 2 tablets as back-up). These 3 containers were packed in a secondary packaging consisting of a white rectangular tub with a lid.

6.5.9 Selection and timing of dose for each volunteer

The initial dose were taken as close as possible to the onset of moderate to severe menstrual cramping pain, as assessed by the volunteer. Study medication was taken with 250 ml water either one hour before or after ingestion of food. The actual times of IP

ingestion were documented in the diary, and the statistical analysis was done using the actual times that each dose was taken. Eight-hourly daily dosing was planned to continue for 24 hours, and thereafter on an as needed basis, dependant upon self-administration of rescue medication.

6.5.10 Blinding

The randomisation code was generated and filed in such a manner as to ensure that all study personnel excluding the pharmacists, remained blinded until study unblinding.

6.5.11 Efficacy data assessed

6.5.11.1 Primary efficacy endpoints

Pain intensity was recorded at baseline (0 hours), 30, 60 and 90 minutes and at 2, 3, 4, 5, 6, 7, 8, 12 and 24 hours.

Pain intensities were defined as follows:

0 = no pain, 1 = mild pain, 2 = moderate pain, 3 = severe pain.

6.5.11.2 Secondary efficacy endpoints

The following secondary efficacy parameters were calculated:

Total (time-weighted) pain relief from 0 to 8 hours (TOTPAR₀₋₈), 0 to 12 hours (TOTPAR₀₋₁₂) and 0 to 24 hours (TOTPAR₀₋₂₄), post first dose of study medication. Total pain relief was recorded at baseline (0 hours), 30, 60 and 90 minutes and at 2, 3, 4, 5, 6, 7, 8, 12 and 24 hours. Total pain relief will be defined as follows:

0 = none, 1 = a little, 2 = moderate, 3 = a lot, 4 = complete.

- Pain intensity difference (PID) from baseline.
- Summed pain intensity difference score from 0 to 12 hours (SPID₀₋₁₂) and 0 to 24 hours (SPID₀₋₂₄).
- Percentage of volunteers who required rescue medication.
- Time to ingestion of first dose of rescue medication.
- Percentage of IP used in each treatment period.

- Global evaluation of IPs. This was assessed at the end of the 72-hour treatment period or at early termination as follows:
1 = poor, 2 = good, 3 = excellent.

6.5.12 Safety assessments

Safety and tolerability were assessed in terms of the number, severity and relationship to the IP to the adverse events, as well as any changes in physical examination findings between visit 2 and visit 6 or changes in clinical laboratory values between visit 2 and visit 6. These were examined separately for each treatment group.

6.5.12.1 Recording of adverse events and concomitant medication

Occurrences of AEs were assessed throughout the study. The volunteers recorded adverse events and concomitant illnesses in the study diaries during and in between treatments. The relationship of the adverse event to the study medication was assessed.

6.5.12.2 Physical examination

A physical examination was performed on each volunteer at screening and again at the post-study follow-up examination or upon withdrawal. Values outside the normal ranges were, however reviewed in the clinical context and in regard to other relevant investigations and also in light of the specific IPs administered.

6.5.12.3 Measurement of vital signs and body temperature

Vital signs, pulse, systolic and diastolic blood pressure were measured in the supine position (after 5 minutes rest) and standing after 3 minutes at screening, and visits 1, 2, 3, 4, 5, and post study follow-up (visit 6). Body temperature was measured at all visits at the same time as vital signs.

6.5.12.4 Laboratory investigations

Blood and urine samples were analysed as part of the screening procedures and re-evaluated for post-study follow-up or upon withdrawal. The following safety laboratory parameters were assessed:

Haematology:	leucocytes, erythrocytes, haemoglobin, haematocrit, MCV, MCH, MCHC, platelets, neutrophils, eosinophils, lymphocytes, monocytes and basophils.
Serum biochemistry:	sodium, potassium, urea, creatinine, total protein, albumin, total bilirubin, conjugated bilirubin, ALP, GGT, AST, ALT and glucose (random, at screening only)
Urine analysis:	Specific gravity, pH, protein, glucose, ketones, bilirubin, blood and urobilinogen (Bayer 10SG Multistix®).
Drugs of abuse screen:	Urine – 9 panel Medtox Diagnostics® [tricyclic antidepressants, barbiturates, methadone, benzodiazepines, cannabinoids, opiates, amphetamines, cocaine metabolites, phencyclidine (at screening visit only)]
Alcohol saliva test	Alco-Screen® (at screening visit only)
Pregnancy test	Serum β hCG at screening and post-study visit

Qdot Pharma's laboratory performed all laboratory investigations. The reference ranges were provided and filed in the Clinical Trial Manual (Investigator's Study File) together with the descriptions of the laboratory methods used. All samples were properly labelled by the site staff for correct identification at the laboratory.

The investigator assessed the relevance of each laboratory value (at screening and post-study) outside the normal ranges. He or she wrote NCR (not clinically relevant) or CR (clinically relevant) on the printed laboratory report. The investigator recorded any clinically relevant abnormal value in the CRFs. Post-study laboratory investigations indicating values outside the normal ranges were repeated to establish if and when those values returned to within the normal ranges.

Values outside the normal ranges were, however, reviewed in the clinical context, and in regard to other relevant investigations and also in light of the specific IPs administered,

before a decision was taken to repeat the investigation(s) in question. All clinically relevant changes from screening that was observed post-study were recorded in the CRFs.

6.5.13 Data management

All data management activities were used for all these activities is iVal which is the validated performance system. Adverse event data were coded using MedDRA version 10.1. Previous and concomitant medication taken at any stage during the study was coded using WHO-DD version 2007, quarter 3.

6.6 Statistical and analytical plans

A single statistical analysis was performed at the end of the study.

6.6.1 General

All calculations of area under the curve of pain intensity were performed using WinNonlin Pro version 5.0.1. Extraction of data from the database, as well as other statistical analyses was performed using SAS® (Version 8.2, SAS Institute Inc., Cary, NC, USA). Throughout the analyses, continuous data were summarised using descriptive statistics where the following parameters were reported: Categorical data was presented using frequencies i.e. number and percentage of volunteers (using the number of volunteers without missing data in the calculation). All statistical analyses were interpreted at the 5 % significance level (2-tailed). Baseline was defined as day 1, 0 hours and end point as day 3, 72 hours.

6.6.2 Missing values

Missing values for the primary efficacy variable at any time-point were handled as missing.

6.6.3 Selection of volunteers for statistical analysis

This was a cross-over study where each volunteer received all 4 treatments in the 4 treatment periods. Therefore, if a volunteer was excluded from one of the populations, it resulted in the exclusion of that volunteer for that period only and not from the entire study, i.e. if a volunteer took study medication for 3 of the 4 treatment periods, the volunteer was excluded from the safety population for the treatment period where she did not take any study medication, but was still included in the other 3 treatment periods.

The following populations were identified for the purpose of the statistical analyses.

6.6.4 Safety population

All volunteers who were randomised and received at least one dose of randomised treatment were included in the safety population.

6.6.5 Intention to treat (ITT) population

This analysis was considered primary. All randomised volunteers meet these criteria were included in the ITT population:

- received the randomised IP at baseline and
- have an evaluable area under the curve measurement for the primary efficacy variable, SPID₀₋₈.

An evaluable area was defined as having at least 4 pain intensity ratings post-dose. A volunteer was included if the volunteer had only 2 ratings of which one was at baseline and the other at 8 hours post-dose.

6.6.6 Per Protocol (PP) population

Volunteers were included in the PP population if they:

- were valid for the ITT population
- did not have major protocol violations
- were withdrawn from the study due to lack of efficacy or an adverse event related to the IP.

Protocol violations were defined as major if they had an influence on the efficacy outcome of the treatment of the volunteer. Major protocol violations did not result in the volunteer's exclusion from the PP population. If a volunteer had a major protocol violation for a specific treatment period then that volunteer was excluded for that treatment period only and included in the PP analysis for the other treatment periods.

6.6.7 Baseline characteristics

Study completion, study withdrawals, exclusions and violations were summarised and the reasons for withdrawals, exclusions, violations and volunteer disposition were listed. A one-sample t-test was used to test for any statistically significant differences in continuous demographic and disease factors at baseline.

6.6.8 Previous and concomitant medication

Previous and concomitant medication taken at any stage during the study was coded using the WHO Drug Dictionary (WHO-DD version 2007, quater1).

6.6.9 Previous and current illnesses

Medical history, surgical history as well as concomitant illnesses were coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 10.1.

6.6.10 Efficacy data

The efficacy analysis was performed for both the ITT and PP populations, with the ITT analysis considered primary.

6.6.10.1 Primary efficacy endpoints

In order to assess the overall analgesic effect, timed ratings of pain intensity were used to calculate the pain intensity difference (PID) by subtracting the pain intensity at each recording from the baseline score. An 8-hour, 12-hour and 24-hour summed pain intensity difference (SPID) score was calculated from the area under the pain time-effect curve by using the trapezoidal rule.

Both PID and SPID scores were analysed by an ANCOVA model including treatment group and period as main fixed effects, volunteer as random factor and baseline pain intensity as a covariate.

6.6.10.2 Secondary efficacy endpoints

Timed ratings of the pain relief were used to calculate the total pain relief score at 8 hours (TOTPAR₀₋₈), 12 hours (TOTPAR₀₋₁₂) and 24 hours (TOTPAR₀₋₂₄). This was done using the area under the pain relief-time curve calculated by using the trapezoidal rule.

The mean TOTPAR₀₋₈, TOTPAR₀₋₁₂ and TOTPAR₀₋₂₄ were presented descriptively as well as graphically for the treatment groups at the different intervals. Furthermore, TOTPAR₀₋₈, TOTPAR₀₋₁₂ and TOTPAR₀₋₂₄ were analysed by an ANCOVA model including treatment group and period as main fixed effects, volunteer as random factor and baseline pain intensity as a covariate.

Time to rescue medication was analysed by using the Kaplan-Meier estimator and the Cox regression model to test for statistical significance between the treatment groups.

Global evaluation of the IP was done by the volunteers at 72 hours or early termination visit. The mean global evaluation score was presented descriptively for the treatment groups and was analysed using the Fisher's exact test.

6.6.11 Adverse events

Analyses of AEs included AEs that started prior to the first administration of the randomised IP, during the period of randomised IP administration, and after the cessation of the IP. A frequency overview of all adverse events was presented per treatment group for the safety population. Relationship to the IP was presented for all adverse events per treatment group. AEs where the relation was detailed as highly probable, probable, possible or improbable were classified as "Related". Other classifications were "Not related" and "Not assessable" and correlated with the investigator's description.

6.6.12 Determination of sample size

A sample size of 66 was required to detect a mean difference of at least 4.0 in time-weighted SPID₀₋₈ with a standard deviation of 9 units [assuming 95 % power and a type 1 error of 0.05 (for a 2-sided test)]. Therefore a total of 100 volunteers were enrolled to ensure that 66 volunteers complete the study (Committee for Proprietary Medicinal Products, 2003).

6.7 Results

6.7.1 Study volunteers

There were 15 major protocol violators. Other protocol violations were not considered to have any effect on the clinical or statistical outcomes.

6.7.1.1 Disposition of volunteers

In Appendix E, it can be seen that out of the 155 volunteers screened for the study, 100 were randomised to treatment. Eighty one volunteers completed the study. Of the 19 volunteers that did not complete the study, 4 volunteers dropped out due to adverse events, 11 volunteers due to protocol violations, 1 volunteer was lost to follow-up and 3 volunteers withdrew consent.

6.7.2 Data sets analysed

Efficacy analyses were performed on both the ITT and the PP populations. The ITT population was regarded as the primary efficacy population with regard to these analyses. Six volunteers did not take any IP and were excluded from the safety population. Additional to these, 13 volunteers did not take the IP for certain treatment periods. These volunteers were excluded from the safety population for the treatment periods mentioned above, but they were included in the safety population for their other treatment periods. Three volunteers did not have an evaluable area under the curve measurement for the primary efficacy variable, SPID₀₋₈, for treatment periods 1, 4 and 1. Additional to the volunteers excluded from the safety population and therefore already excluded from the ITT population, 2 volunteers were excluded from the ITT population for treatment period 1.

More volunteers were excluded from the PP population due to major protocol violations. The number of volunteers included in the different analysis populations is summarised per treatment group in Table 6.2, where the various populations are defined in terms of the percentages of the total number of volunteers randomised.

Table 6.2: Population analysis of the volunteers

	Triple FDC		Triple FDC/Placebo		Double FDC		Placebo	
	N	(%)	N	(%)	N	(%)	N	(%)
Randomised volunteers	100	100.0	100	100.0	100	100.0	100	100.0
Safety Population	88	88.0	89	89.0	89	89.0	86	86.0
ITT Population	88	88.0	89	89.0	88	88.0	85	85.0
PP Population	84	84.0	83	83.0	84	84.0	82	82.0

6.7.2.1 Demographic variables

All the randomised volunteers were Caucasian females. They had a mean age of 28 years, BMI of 24.56 kg/m², height of 168.3 cm and weight of 69.66 kg. The p-value was obtained from one-sample t-test. There were statistical significant differences for the distribution of all the variables between the randomised volunteers. This did not have any effect on the efficacy analysis of the different treatments.

6.7.2.2 Previous and concomitant medication

Six volunteers took anti-inflammatory and anti-rheumatic products during the study. These were regarded as protocol violations and it was decided to withdraw these volunteers from the study. The remaining 4 volunteers that took anti-inflammatory and anti-rheumatic products as well as the remaining 27 volunteers that took analgesics during the study did so under the supervision of the investigator and at times when it did not influence the analgesic effect of the IP.

6.7.2.3 Medical history of primary dysmenorrhoea

Mean onset age of primary dysmenorrhoea was found to be 16 years and the number of days of menstrual cramping 3 days. Statistical significant differences exist for the

distribution of both onset age of primary dysmenorrhoea and the number of days of menstrual cramping per cycle.

6.7.2.4 Pain intensity rating at screening

All volunteers experienced pain associated with menstruation that reached either moderate or severe intensity. Sixty one volunteers experienced moderate pain while 39 experienced severe pain during menses. None of the volunteer experienced “no pain or mild pain” during the menses.

6.7.2.5 Medical or surgical history and concurrent illnesses

No clinically relevant surgical history and past or ongoing current medical conditions were recorded at screening.

6.7.3 Efficacy results

The p-values were obtained from an ANCOVA with treatment group, sequence effect and period as main fixed effects, volunteer as random factor and baseline pain intensity as a covariate. The p-value for global evaluation where the p-value was obtained from a Fisher's exact test.

6.7.3.1 Primary evaluation of efficacy

The SPID₀₋₈ was calculated for each volunteer per treatment group. The mean PID by timed interval and the mean summed pain intensity difference score from 0 to 8 hours (SPID₀₋₈) is summarised in Table 6.3. Data for the ITT population are presented in this section, since this did not vary significantly from that for the PP population. Additionally, p-values due to between treatment group comparisons are presented in Table 6.4.

The results in Table 6.3 show that for the PID score at the 1.5 hours, 2 hours, 3 hours and 6 hours intervals, statistically significant differences between the different treatment groups exist. It also shows statistically significant ($p < 0.05$) differences for the mean SPID₀₋₈ score between the different treatment groups.

6.7.3.2 Secondary evaluation of efficacy

The mean TOTPAR scores from 0 to 8 hours (TOTPAR₀₋₈), from 0 to 12 hours (TOTPAR₀₋₁₂) and from 0 to 24 hours (TOTPAR₀₋₂₄) are summarised in Table 6.5. The differences between the different treatment groups for TOTPAR are shown in Figure 6.1. Table 6.5 shows that statistically significant differences between the different treatment groups exist for the total pain relief score calculated from 0 to 8 hours, 0 to 12 hours and 0 to 24 hours. The results in Table 6.6 show the p-values as a result of between group comparison and exactly where the statistically significant differences exist when the different treatment groups are compared to each other.

6.7.3.3 Pain intensity difference (PID)

The mean PID by timed interval up to 8 hours post-dose is summarised in Table 6.3. The PID score up to 24 hours post-dose is summarised in Table 6.7 and shown in Figure 6.2. Additionally, p-values as a result of between treatment group comparisons are presented in Table 6.8.

Table 6.3: Pain intensity difference (PID) and summed pain intensity difference (SPID₀₋₈) from 0 to 8 hours in the ITT Population

Variable	Time points (h)	Triple FDC	Triple FDC/ Placebo	Double FDC	Placebo	p-value
		(N=88)	(N=89)	(N=88)	(N=85)	
Mean PID score	0.5	-0.3	-0.2	-0.4	-0.2	0.1609
	1.0	-0.9	-0.8	-0.8	-0.6	0.1257
	1.5	-1.2	-1.1	-1.1	-0.8	0.0032*
	2.0	-1.5	-1.4	-1.3	-1.0	0.0007*
	3.0	-1.5	-1.5	-1.3	-1.1	0.0125*
	4.0	-1.5	-1.6	-1.4	-1.2	0.0769
	5.0	-1.6	-1.5	-1.4	-1.3	0.0598
	6.0	-1.5	-1.3	-1.1	-1.1	0.0253*
	7.0	-1.3	-1.1	-1.0	-1.0	0.1088
	8.0	-1.2	-1.0	-0.9	-0.9	0.1805
Mean SPID ₀₋₈		-11.043	-9.394	-8.964	-7.956	0.0063*

* Statistically significant

Table 6.4: Between treatment comparison for PID and SPID₀₋₈ in the ITT Population

Timepoints (h) Variable	p-value					
	Triple vs Triple /Placebo	Triple vs Double	Triple vs Placebo	Triple vs /Placebo Double	Triple FDC /Placebo vs Placebo	Double FDC vs Placebo
0.5	0.4386	0.3899	0.2493	0.0563	0.9135	0.0726
1.0	0.2553	0.5038	0.0157*	0.5316	0.2010	0.0451*
1.5	0.2851	0.3410	0.0003*	0.9982	0.0054*	0.0056*
2.0	0.3834	0.0986	<.0001*	0.3823	0.0017*	0.0125*
3.0	0.5138	0.0727	0.0034*	0.2341	0.0094*	0.1250
4.0	0.7563	0.2433	0.0229*	0.3382	0.0237*	0.2116
5.0	0.4709	0.0783	0.0075*	0.3192	0.0763	0.4592
6.0	0.2305	0.0136*	0.0087*	0.2293	0.1281	0.7720
7.0	0.1788	0.0315*	0.0489*	0.4055	0.4753	0.7875
8.0	0.1124	0.0876	0.0549	0.8965	0.7438	0.9869
Mean SPID ₀₋₈	0.0422*	0.0162*	0.0015*	0.6911	0.0975	0.2572

Statistically significant

Table 6.5: Total pain relief (TOTPAR) in the ITT Population

Variable	Measure	Triple FDC	Triple FDC/ Placebo	Double FDC	Placebo	p-value
		(N=88)	(N=89)	(N=88)	(N=85)	
TOTPAR ₀₋₈	Mean	18.826	16.119	15.637	13.633	0.0099
	SD	12.783	9.431	10.014	9.910	
TOTPAR ₀₋₁₂	Mean	29.178	25.286	25.105	21.751	0.0182
	SD	18.303	14.124	15.545	15.613	
TOTPAR ₀₋₂₄	Mean	63.852	55.815	56.504	49.571	0.0247
	SD	34.554	28.974	31.399	32.153	

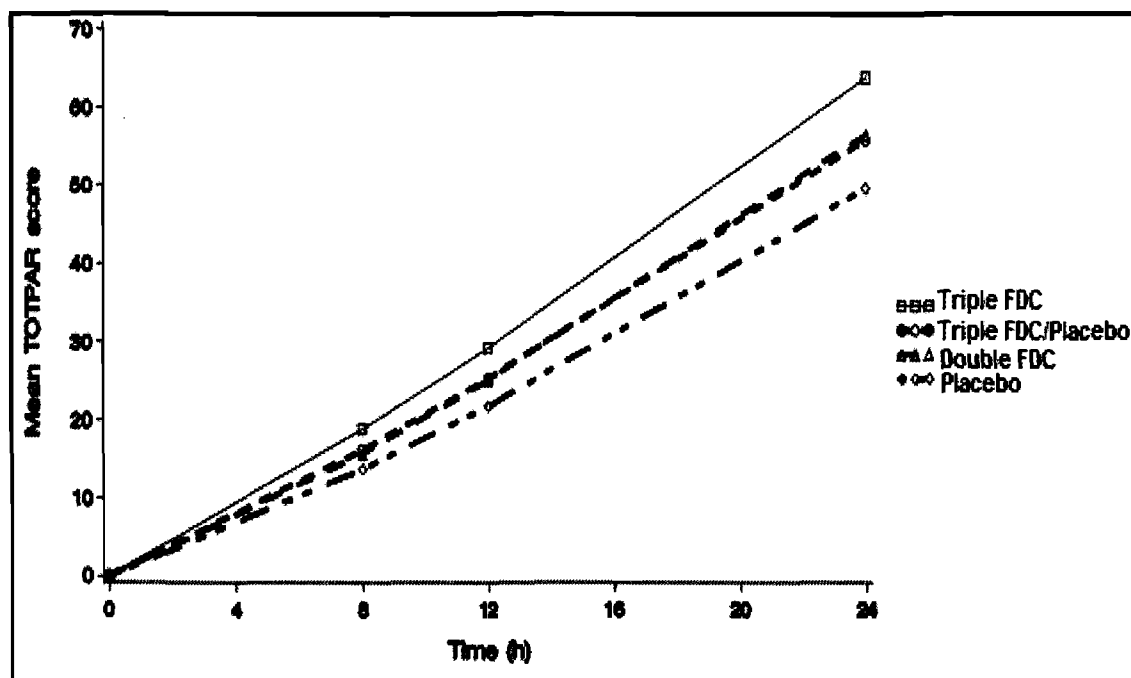


Figure 6.1: Mean TOTPAR score over time for the ITT Population

Table 6.6: TOTPAR between treatment comparison for ITT Population

p-value						
Variable	Triple vs Triple/ Placebo	Triple vs Double	Triple vs Placebo	Triple/ Placebo vs Double	Triple / Placebo vs Placebo	Double vs Placebo
TOTPAR ₀₋₈	0.1191	0.0632	0.0023*	0.7520	0.0386*	0.1024
TOTPAR ₀₋₁₂	0.1355	0.1023	0.0037*	0.9316	0.0655	0.1215
TOTPAR ₀₋₂₄	0.0982	0.1354	0.0069*	0.8559	0.1457	0.1494

Table 6.7: Pain intensity difference (PID) for 12 and 24 hours post-dose in the ITT Population

		Triple FDC	Triple FDC/ Placebo	Double FDC	Placebo	p-value
Variable	Time points (h)	N=88	N=89	N=88	N=85	
Mean PID score	12.0	-1.6	-1.4	-1.6	-1.2	0.0214*
	24.0	-1.7	-1.6	-1.5	-1.4	0.1311

*Statistically significant

Table 6.8: Between treatment comparison for PID for 12 and 24 hours post-dose in the ITT Population

Timepoints	p-value					
	Triple vs Triple/ Placebo	Triple vs Double	Triple vs Placebo	Triple/ Placebo vs Double	Triple/ Placebo vs Placebo	Double vs Placebo
12 hours	0.0720	0.3509	0.0055*	0.3131	0.2232	0.0353*
24 hours	0.1548	0.0630	0.0316*	0.7462	0.4695	0.7691

Statistically significant

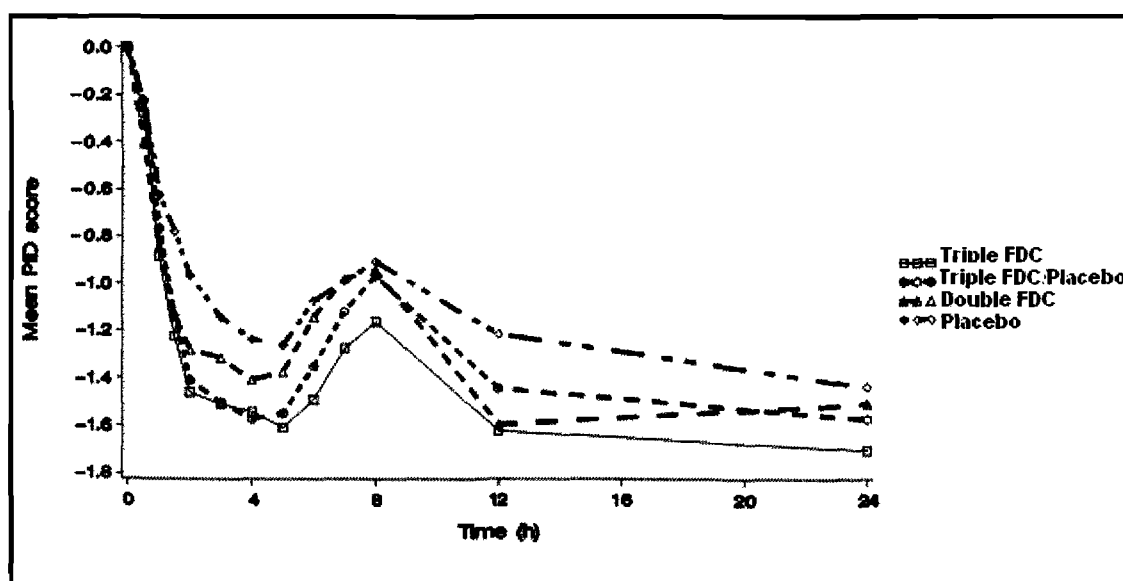


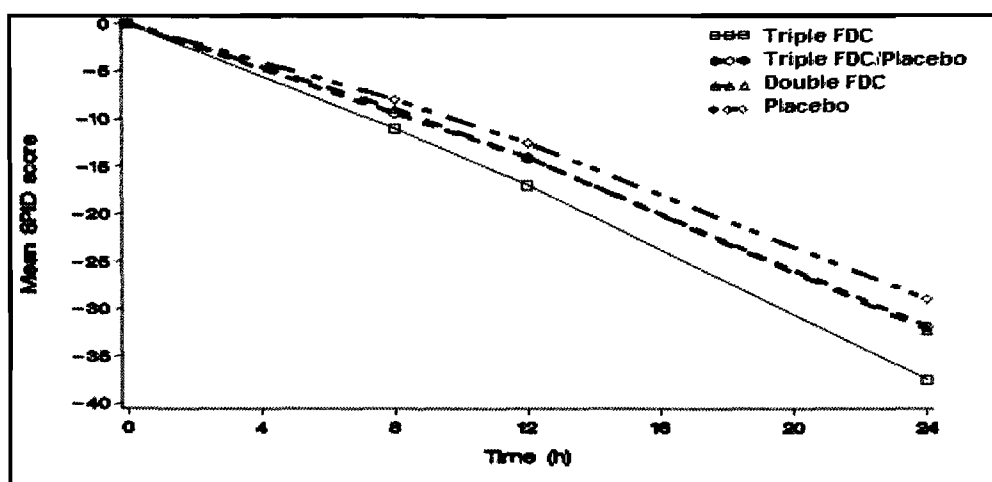
Figure 6.2: Mean PID score over time in the ITT population

6.7.3.4 Summed pain intensity difference (SPID)

The mean summed pain intensity difference score from 0 to 8 hours (SPID₀₋₈) is already summarised in Table 6.3. The SPID score up to 24 hours post-dose is shown in Figure 6.3 and summarised in Table 6.9. Additionally, p-values as a result of between treatment group comparisons are presented in Table 6.10.

Table 6.9: Summed pain intensity difference (SPID) in ITT population

Measure	Triple FDC	Triple FDC/ Placebo	Double FDC	Placebo	p-value
	(N=88)	(N=89)	(N=88)	(N=85)	
Mean SPID ₀₋₁₂	-16.987	-14.073	-14.063	-12.533	0.0085*
Mean SPID ₀₋₂₄	-37.489	-31.870	-32.221	-28.960	0.0131*

**Figure 6.3:** Mean SPID score over time in the ITT population**Table 6.10:** Between treatment comparison of SPID in the ITT population

Variable		p-value ¹				
Mean	Triple vs Triple/ Placebo	Triple vs Double	Triple vs Placebo	Triple/ Placebo vs Double	Triple/ Placebo vs Placebo	Double vs Placebo
SPID ₀₋₁₂	0.0235*	0.0188*	0.0028*	0.9747	0.2566	0.3343
SPID ₀₋₂₄	0.0264*	0.0286*	0.0056*	0.8577	0.3580	0.3749

* Statistically significant

6.7.4 Volunteers who required rescue medication

Table 6.11 shows the number and percentage of volunteers in the ITT Population who required rescue medication. The percentage of volunteers who required rescue medication in the ITT population is shown in Figure 6.4.

The Kaplan-Meier estimator in Figure 6.5 is used to compare the different treatment groups with regard to the time to rescue medication for each volunteer who took rescue medication up to 72 hours. Each downward step on the graph represents an event time (when a volunteer took rescue medication).

Table 6.11: Number of volunteers who required rescue medication in the ITT population

Triple (N=88)	FDC	Triple /Placebo (N=89)	FDC	Double (N=88)	FDC	Placebo (N=85)
5		6		7		10

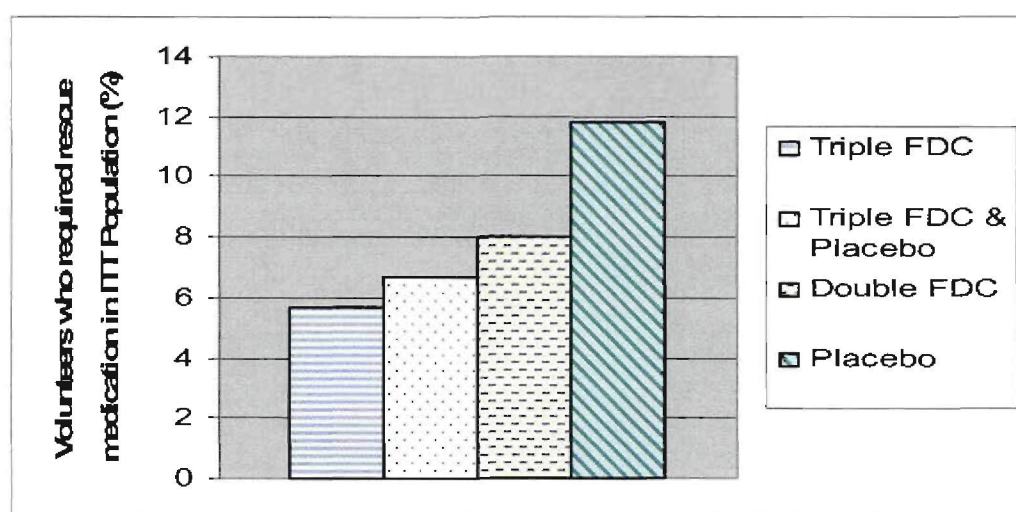


Figure 6.4: Columns showing the percentage of volunteers who required rescue medication in the ITT population

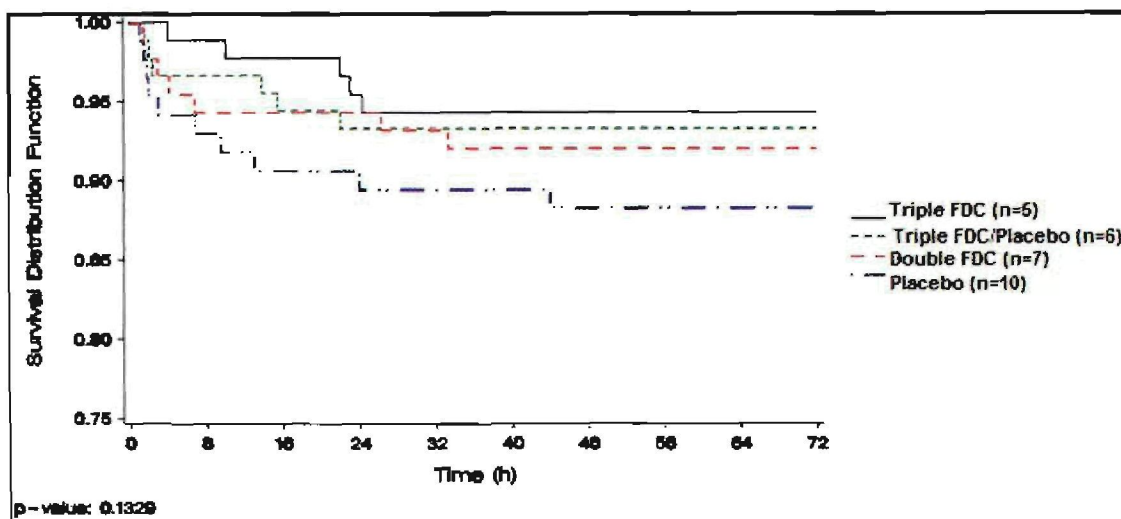


Figure 6.5: Time to rescue medication: Kaplan-Meier estimator in the ITT population

6.7.5 Global evaluation of the IP

The global evaluation score of the IP is summarised per treatment group in Table 6.12. The p-value was obtained from a Fisher's exact test.

Table 6.12: Mean global evaluation score in the ITT population

	Triple FDC	Triple FDC / Placebo	Double FDC	Placebo	
Measure	n	n	n	n	p-value
Missing	3	0	4	2	0.0036*
1 – Poor	7	17	18	28	
2 – Good	50	47	40	40	
3 – Excellent	28	25	26	15	
Total	88	89	88	85	

* Statistically significant

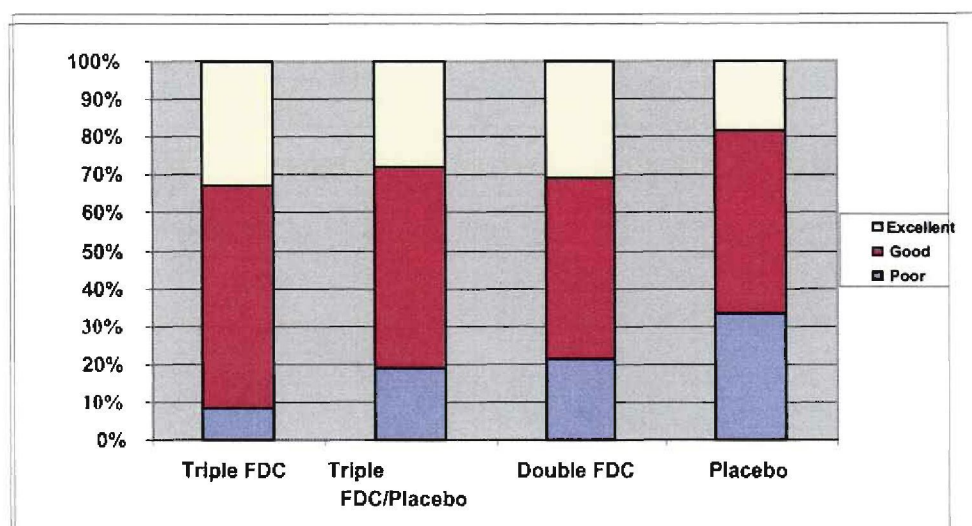


Figure 6.6: Volunteer mean global evaluation score (%) in the ITT Population

The percentage of the ITT Population for the comparison of the mean global score evaluation are shown in Figure 6.6. Additionally, p-values as a result of between treatment group comparisons are presented in Table 6.13.

Table 6.13: Between treatment comparison of mean global evaluation score in ITT population

p-value					
Triple vs Triple/Placebo	Triple vs Double	Triple vs Placebo	Triple /Placebo vs Double	Triple/Placebo vs Placebo	Double vs Placebo
0.1122	0.0472*	0.0001*	0.8102	0.0633	0.0810

6.7.6 Efficacy conclusions

The overall analgesic effect was assessed the pain intensity difference score (PID). Of all the treatment groups, only Triple FDC Capsules showed better results for PID than Placebo at all time-points except at 0.5 and 8 hours.

The graph representing the mean PID score over time in Figure 6.3 shows that pain got worse around the 8-hours post-dose time-point in all treatment groups.

For the efficacy variable, summed pain intensity difference (SPID), Triple FDC Capsule was significantly better than the other treatment groups at all time point.

The total pain relief experienced was significantly better for the Triple FDC Capsules group than for the Placebo group. Total pain relief in the Triple FDC Capsules/Placebo group and the group that took the Double FDC capsules showed similar results, while the results for the Placebo group showed the least pain relief.

Fewer volunteers in the Triple FDC Capsules, Triple FDC Capsules/Placebo and Double FDC capsule groups required rescue medication compared with the Placebo group. The Kaplan-Meier curve in Figure 6.5, also shows that the rate at which volunteers in the Placebo group required rescue medication was greater than for the other treatment groups. Significantly more volunteers receiving Triple FDC Capsules rated their study medication as good or excellent compared to the other treatment groups.

6.7.7 Safety evaluation

6.7.7.1 Extent of exposure

A summary of the number of volunteers to whom IP and rescue medication was dispensed and returned per treatment period is documented in Table 6.12. No dosage or concentration adjustments were made to the IP prior to their dosing for any volunteers.

Table 6.14: IP and rescue medication dispensed and returned in safety population

	Triple FDC		Triple/Placebo		Double FDC		Placebo		Unscheduled	
	n	%	n	%	n	%	n	%	n	%
IP										
Dispensed	88	100	89	100	89	100	86	100	9	88
Returned	88	100	89	100	89	100	86	100	9	
Rescue medication										
Dispensed	88	100	89	100	89	100	86	100	9	88
Returned	88	100	89	100	89	100	86	100	9	88

Major protocol violations with respect to the dosing of the IP are summarised in Table 6.15.

6.7.8 Adverse Events

6.7.8.1 Brief summary of adverse events

A total of 148 adverse events were reported during the entire study period in 74 of the 100 volunteers. Of these, 21 events started prior to the first administration of the IP. The total occurrence of adverse events during the study is summarised in Table 6.16.

Related events include all adverse events where the relationship to the IP was regarded as being highly probable, probable, possible or improbable by the investigator.

The data in Table 6.16 suggest that the distribution of adverse events were almost similar in the four different treatment groups, with the largest proportion occurring in the Triple FDC group. Adverse events graded as severe in intensity were experienced in all treatment groups with the largest proportion occurring in the Placebo group. Five serious adverse events occurred in 5 volunteers (pregnancy; regarded as not related to the IP). One volunteer did not take any study medication and was excluded from the safety population. One volunteer took study medication for treatment period 1 only. She took the IP even though the pregnancy test was positive.

Table 6.15: Major protocol violations with respect to the dosing of the IP for all randomized volunteers

Treatment Period	Violation	Number of volunteers
Period 1	Took second dose of IP before 8 hour pain intensity rating	3
	Volunteer took medication even though pregnancy test was positive (Observation 2)	1
	Volunteer took study medication even though her pain was not moderate to severe	1
Period 2	Volunteers received incorrect treatments when unscheduled visits occurred	6
	Took second dose of IP before 8 hour pain intensity rating	8
	Volunteers received incorrect treatment by error	1
	Volunteers received incorrect treatments when unscheduled visits occurred	8
Period 3	Took second dose of IP before 8 hour pain intensity rating	3
	Volunteer took study medication even though her pain was not moderate to severe	2
	Volunteers received incorrect treatment by error	1
	Volunteers received incorrect treatments when unscheduled visits occurred	8
Period 4	Took second dose of IP before 8 hour pain intensity rating	1
	Volunteers received incorrect treatment by error	1
	Volunteers received incorrect treatments when unscheduled visits occurred	8
Unscheduled	Volunteers received incorrect treatments when unscheduled visits occurred	8

Table 6.16: Summary of Adverse Events in the Safety Population

	Triple FDC (N=88)	Triple / Placebo (N=89)	Double FDC (N=89)	Placebo (N=86)
Total number of volunteers with at least one adverse event	25	21	23	24
Total number of adverse events	37	27	29	34
Total number of severe adverse events	11	9	11	16
Total number of serious adverse events (pregnancy)	0	0	1	2
Total number of adverse events related to the IP	13	10	12	15
Total number of adverse events leading to discontinuation	1	0	0	1

Adverse events regarded as related to IP were experienced in all treatment groups with the largest proportion occurring in the Placebo group. Two adverse events led to withdrawal from the study, 1 in the Triple FDC Capsules group– asthma and 1 in the Placebo group – pregnancy. The most common body system affected by adverse events was infections and infestations with nasopharyngitis and influenza featuring most prominently. Thereafter, adverse events relating to the nervous system were most frequent in most of the dosing groups, followed by disorders related to the gastrointestinal system as well as injury, poisoning and procedural complications. This information is summarised in Table 6.17.

6.7.9 Analysis of adverse events

6.7.9.1 Deaths

No deaths or other serious adverse events occurred during the study or during the post-treatment follow-up period.

6.7.9.2 Clinical Laboratory Evaluation

The clinical laboratory tests (haematology, biochemistry and urinalysis) were assayed by QDot Pharma Laboratory, a Division of Parexel International (South Africa) (Pty) Ltd. No clinically significant abnormal laboratory results were reported for this study. Review of these data over time revealed no clinically significant changes in the spread of data from screening to visit 6 with respect to any of the haematological parameters. No significant changes in the spread of the data were noted for any of the urinalysis parameters. No obvious differences with regard to vital signs were evident in any of the treatment groups.

6.7.9.3 Vital signs

Vital signs that were monitored during the study included supine blood pressure and heart rate, standing blood pressure and heart rate. These were monitored at screening (visit 1) and visits 2, 3, 4, 5, and post-study follow-up (visit 6). Body temperature was also monitored during the study at the same visits as the blood pressure and heart rate measurements. No obvious differences with regard to vital signs were evident in any of the treatment groups.

6.7.9.4 Physical findings

Formal physical examinations were conducted for each volunteer at screening and again at the post-study follow-up visit. No clinically relevant findings were reported at screening. At the follow-up visit, 5 volunteers reported clinically relevant findings. These findings were reported as adverse events and on further follow-up all of these were resolved.

Table 6.17: Adverse Events that started during randomised treatment by System Organ Class in the Safety population

System Organ Class ¹	Triple (N=88)			FDC Capsules			Triple Capsules/Placebo (N=89)			FDC Double (N=89)			capsules Placebo (N=86)		
	n	(m)	%	n	(m)	%	n	(m)	%	n	(m)	%	n	(m)	%
Eye disorders	1	(1)	1.1	1	(1)	1.1	0	(0)	0	0	(0)	0	0	(0)	0
Gastrointestinal disorders	2	(3)	2.3	3	(4)	3.4	1	(1)	1.1	4	(7)	4.7			
General disorders and administration site conditions	1	(1)	1.1	0	(0)	0	0	(0)	0	0	(0)	0	0	(0)	0
Infections and infestations	14	(14)	15.9	13	(13)	14.6	10	(10)	11.2	12	(13)	14.0			
Injury, poisoning and procedural complications	4	(4)	4.5	1	(2)	1.1	3	(3)	3.4	2	(2)	2.3			
Musculoskeletal and connective tissue disorders	2	(2)	2.3	1	(1)	1.1	0	(0)	0	0	(0)	0	0	(0)	0
Nervous system disorders	7	(10)	8.0	2	(3)	2.2	9	(14)	10.1	8	(9)	9.3			
Pregnancy, puerperium and perinatal conditions	0	(0)	0	0	(0)	0	1	(1)	1.1	2	(2)	2.3			
Respiratory, thoracic and mediastinal disorders	1	(1)	1.1	0	(0)	0	0	(0)	0	0	(0)	0	0	(0)	0
Skin and subcutaneous tissue disorders	1	(1)	1.1	2	(2)	2.2	0	(0)	0	1	(1)	1.2			
Vascular disorders	0	(0)	0	1	(1)	1.1	0	(0)	0	0	(0)	0	0	(0)	0
All combined	25	(37)	28.4	21	(27)	23.6	23	(29)	25.8	24	(34)	27.9			

N = Total no. of the volunteers in the safety population per treatment group; n = No. of volunteers; m = No. of mentions

6.7.9.5 12-Lead ECG findings

12-Lead ECG examinations were conducted for each volunteer at screening and again at the post-study follow-up visit. No clinically relevant abnormal 12-Lead ECG findings were reported for this study. One volunteer, had an abnormal ECG result at screening. This abnormality was not regarded as clinically relevant.

6.8 Safety conclusions

Triple FDC Capsules did not have relevant effects on the safety laboratory haematology, biochemistry or urinalysis parameters determined in this study. 12-Lead ECG recordings did not reveal any significant arrhythmias. One ECG, performed at screening, showed a right bundle branch blockade. This was regarded as not clinically relevant. No obvious differences with regard to vital signs were evident in any of the treatment groups.

6.9 Discussion

Placebo is not “inert”. Almost 50 % of participants in the Placebo group experienced satisfactory pain relief from 0 to 8 hours post dose. This is not surprising since grading of pain is subjective and expectation of efficacy evidences itself in perceived pain relief. This observation underscores the importance of placebo-controlled studies in regard to pain-relief comparisons. In other words, any inference about analgesic efficacy of active drugs (single entities or combinations) is only valid when “placebo noise” is “subtracted”. The test model employed had sufficient discriminative power to distinguish between Triple FDC Capsules and Placebo from 0 to 8 hours, and all the other treatments from 8 to 24 hours.

Triple FDC Capsules provided satisfactory pain relief from 0 to 24 hours after taking two capsules 8-hourly, with the two capsule 8-hourly regimen being marginally superior to the one capsule 8-hourly (Triple FDC Capsules/Placebo) and Double combination regimens. The onset of action is faster with the Double FDC.

Triple FDC Capsules (2 capsules) taken 8-hourly, is probably an optimal regimen for this combination. The relatively long elimination half-life of meloxicam did not manifest itself in

the duration of action of Triple FDC Capsules. The study design can be criticised for not exploring the exact contribution of each of the active ingredients of Triple FDC Capsules to pain relief in primary dysmenorrhoea. Paracetamol and NSAIDs, such as meloxicam, are effective in their own right. Importantly, the study did not allow for an assessment of the pain relieving potential of 2.5mg or 5 mg meloxicam only, taken 8-hourly, in primary dysmenorrhoea. However, the main objectives of this exploratory study were to determine whether Triple FDC Capsules is effective in relieving pain associated with dysmenorrhoea, and to gain information in regard to Triple FDC Capsules dose versus response. Both of these objectives have been satisfied.

6.10 Conclusion

All four treatments at all points of measurement provided relief of pain associated with primary dysmenorrhoea. Triple FDC Capsules (2 capsules) taken 8-hourly appears to be an effective and well-tolerated analgesic combination.

The pain model employed has the potential to discriminate between analgesic compounds.

CHAPTER 7

SUMMARY AND CONCLUSIONS

7.1 Summary

Although the understanding of the pain mechanism has improved significantly over the years, much more is yet to be discovered. Broadening of our knowledge is needed to improve basic and clinical research in this field in order to successfully treat and manage pain in millions of people.

Due to the complexities of the process involved in the treatment of pain, many modalities are used to alleviate or reduce pain.

Validation of analytical methods is essential but can also be unnecessarily time consuming during the early stages of product development. Various analytical methods are required at different stages of the development life cycle. Although the requirements for method validation of new drug applications are clear, the same is not true for products in early development. Thus in Chapter 2, a method validation by phase development “qualification” was carried out on the methods developed for testing the capsules. This allowed for specific details of some aspects of method validation studies to be delayed during this stage of early development e.g Intermediate precision: during early development, when methods are typically operated in one laboratory and by few analysts, it is not necessary to determine the intermediate precision of an assay method. By phasing method validation, activities and resources can be optimized while maintaining a good scientific approach to pharmaceutical development.

In Chapter 3, the rat study proved that there was indeed a super additive effect when using paracetamol, codeine phosphate and meloxicam in a fixed-dose combination. These agents in a combination were effective at doses in which when; used in isolation they would not be effective. This phenomenon of increased efficacy of NSAIDs by using paracetamol, paracetamol in combination with NSAIDs and codeine phosphate and paracetamol has previously been observed when these agents were administered in combination. Although no formal safety studies were carried out in our rat studies, the

rats remained in satisfactory condition and no weight loss was observed during or after the study.

The reason for the decrease of codeine phosphate on stability seen in Chapter 4 could not be ascertained. Investigations carried out amongst different samples (including the ones for preformulation) have shown that the reaction is due to the green gelatin capsule shells used. The difference between the sample that still complied (with regards to codeine phosphate) and the one in question is the capsule shell composition and colour. Although the dissolution rate slowed, it was however still within the limits for the requirement of the percentage dissolved in 60 minutes, i.e. 80 %. The exact mechanism or reaction involved between the green gelatin capsules and codeine phosphate could not be established and requires further investigation. There have been reports of possible interactions between codeine and sodium lauryl sulphate. In our case the possibility was eliminated based on the formulations that were explored during the development phase. There was sodium lauryl sulphate in these formulation, including the initial blend of the green gelatin capsules and there was no decrease of codeine phosphate on stability.

In Chapter 5, the study to determine the bioavailabilities of the three ingredients of the Triple FDC capsules was carried out according to a randomised, laboratory-blind, single-dose, three-part cross-over study carried out in fasted healthy male/female caucasian volunteers. Reported inter-ethnic differences in the pharmacokinetics of meloxicam and codeine phosphate was the reason for enrolling only one ethnic group in the study. The bioavailabilities of paracetamol, codeine phosphate and meloxicam, as contained in the Triple FDC capsule were compared with paracetamol and codeine phosphate, as contained in Myprodol® (Adcock Ingram Limited), and meloxicam as contained in Mobic® (Boehringer Ingelheim (Pty) Ltd).

The purpose of the study was not to establish bioequivalence but rather to demonstrate that the three drugs do not affect each others respective pharmacokinetic patterns. The results of the study showed that paracetamol and meloxicam, as present in the Triple

FDC capsules, were comparable with those in the reference products in respect of AUC_{0-t} and C_{max} . However, codeine phosphate was less bioavailable from Triple FDC capsules than from Myprodol®. The reason for this observation can be linked back to the decrease in assay results of codeine phosphate on stability. It can be concluded that this is clearly a formulation issue.

No serious adverse events were reported during the study. Pharmacokinetic studies are particularly important in assessing the clearance of the drug and to anticipate possible accumulation of the parent drug or metabolites and potential drug-drug interactions.

The study conducted in Chapter 6 was a multiple dose, double-blind, double-dummy, randomised, placebo and active-controlled cross-over, proof-of-concept study conducted to determine the safety and efficacy of two different doses of the Triple FDC capsule (10 mg codeine phosphate, 250 mg paracetamol, 2.5 mg meloxicam) versus a Double FDC capsule (250 mg paracetamol and 10 mg codeine phosphate) and placebo in females with primary dysmenorrhoea, Phase II study.

The Triple FDC product was evaluated for analgesic efficacy. It was postulated that a combination of meloxicam, paracetamol and codeine phosphate would act synergistically in providing relief of pain associated with dysmenorrhoea. This postulate was supported by the rat study findings in Chapter 3. Dysmenorrhoea has been shown to be an established model for assessing pain relieving qualities of analgesic candidates with regards to mild to moderate acute pain. To eliminate the inter-subject variability due to the reported inter-ethnic differences in the pharmacokinetics of codeine phosphate and meloxicam, as well as in the pain threshold, only Caucasians were enrolled in this study. Dysmenorrhoea affects more than half of menstruating women. Women have painful menses without demonstrable pelvic abnormalities with dysmenorrhoea.

The main objective of the entire study was to gain more information with regards to dose versus response of the Triple FDC and its safety. This objective was attained. The inclusion criteria did however limit extrapolation of the findings to other pain models.

The study reconfirmed the analgesic effect of paracetamol, meloxicam and codeine phosphate for moderate to severe pain. The pain relief does not seem to last longer when combined with meloxicam. The Kaplan-Meier estimator and the cumulative percentage of patients who required rescue medication showed that volunteers on the Triple FDC took longer before using the rescue medication and the percentage of volunteers who took the rescue medication was the least in this group. This suggested that the Triple FDC has a long duration of action when compared to the other treatment groups. One of the main concerns with acute pain treatment when using NSAIDs is decreased renal function.

However, no clinically significant abnormal laboratory results were observed during the study even with the administration of meloxicam three times a day with a long half-life. Significantly, more volunteers receiving Triple FDC capsules rated their study medication as good or excellent compared to the other treatment groups. Triple FDC capsules were unequivocally superior to Placebo in relieving pain. Triple FDC capsules/Placebo and the Double FDC capsule groups were marginally better than Placebo, but indistinguishable from one another.

The side effects, infections and infestations did not seem to be any less for patients taking 1 Triple FDC versus 2 Triple FDC, i.e. 5 mg versus 2.5 mg meloxicam. This suggests that side effects were not less with a decrease in the dose of meloxicam.

7.2 Conclusion

The results obtained in this study supported our hypothesis of synergistic multimodal analgesia when using NSAIDs, COX-inhibitors and an opiod which was supported by the rat study. Although the contribution of the double combination of paracetamol and codeine phosphate were established, the study could be criticised for not including an assessment of pain relieving potential of the 5 mg meloxicam, on its own, taken 8-hourly. The multimodal analgesia in a fixed dosage form proved to be effective in the treatment on pain associated with dymenorrhoea and holds good promise in the treatment of other pain models.

Since modalities used in the treatment of pain are moving from single-drug trials to fixed-dose combination drug therapies, more research is needed with the fixed dose combination therapy in order to obtain optimum analgesia in different types of pain models.

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APPENDIX A

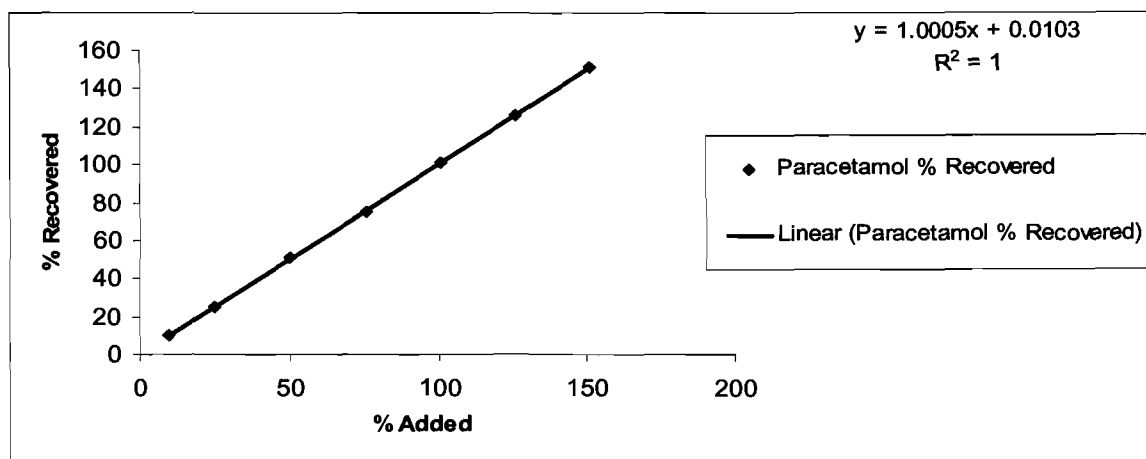


Figure A.1: Linear regression graph for paracetamol to determine linearity and range

Table A.1: Regression statistics of paracetamol results

R squared	0.9999	Lower 95 %	Upper 95 %
Intercept	0.01030	-0.31917	0.339756
Slope	1.00050	0.99697	1.00404

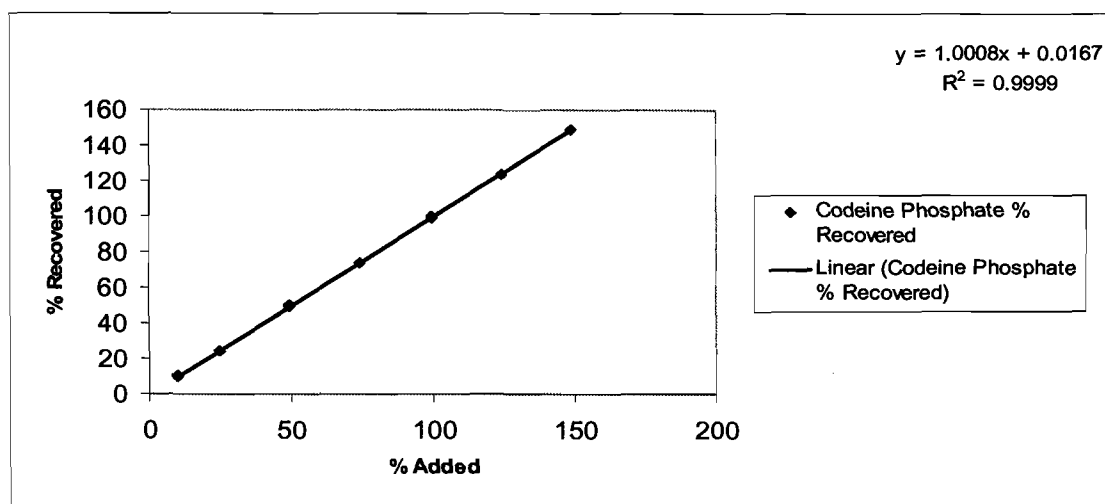
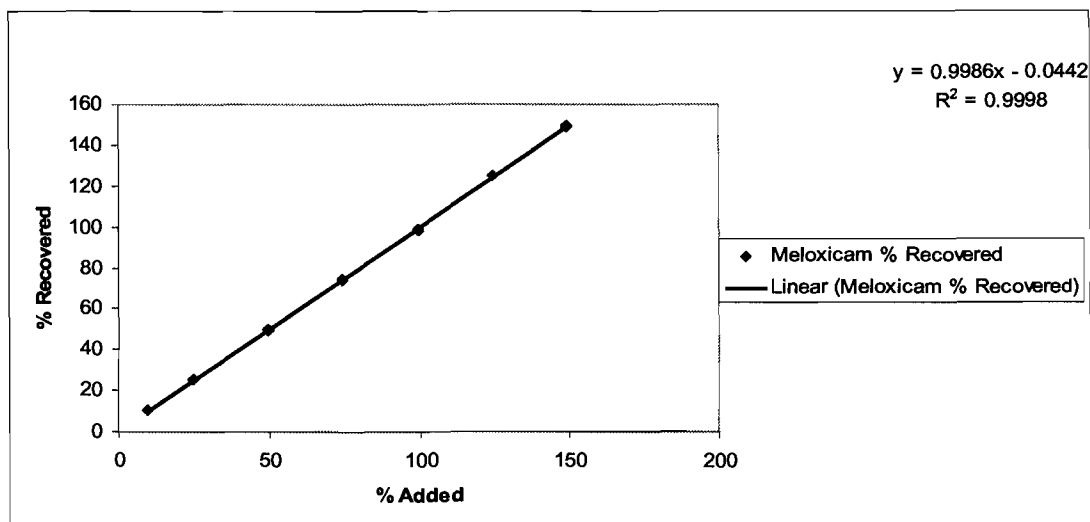


Figure A.2: Linear regression graph for codeine phosphate to determine linearity and range

Table A.2: Regression statistics of codeine phosphate results

R squared	0.9999	Lower 95 %	Upper 95 %
Intercept	0.01673	-0.40796	0.44141
Slope	1.00081	0.99617	1.00544

**Figure A.3:** Linear regression graph for meloxicam to determine linearity and range**Table A.3:** Regression statistics of meloxicam results

R squared	0.9998	Lower 95 %	Upper 95 %
Intercept	-0.04422	-0.69561	0.60717
Slope	0.99864	0.99156	1.00572

APPENDIX B

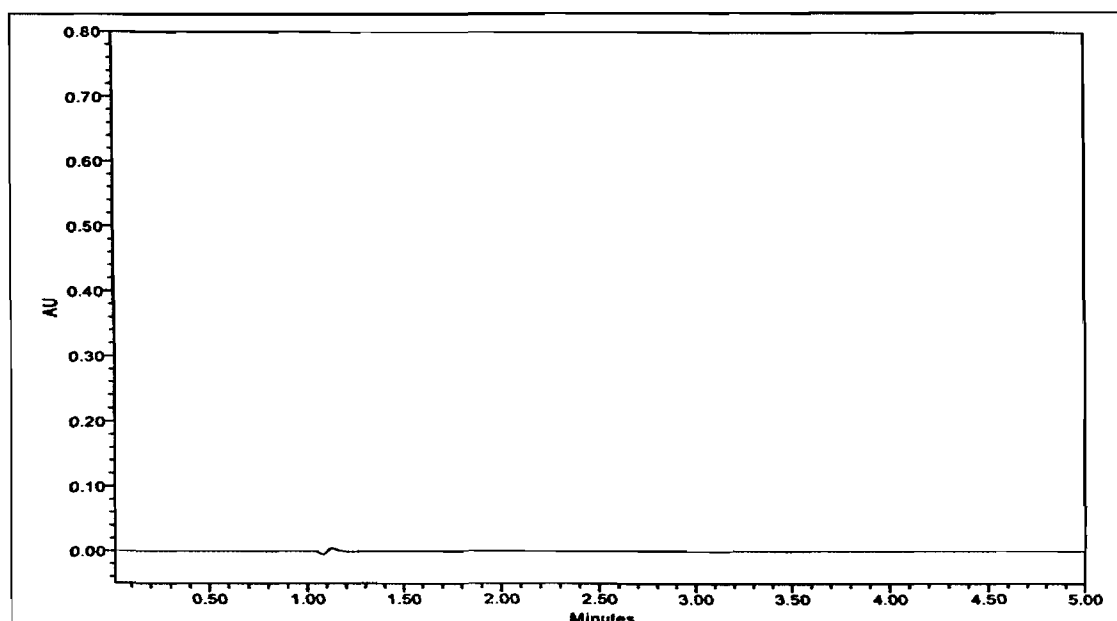


Figure B.1: Placebo Chromatogram

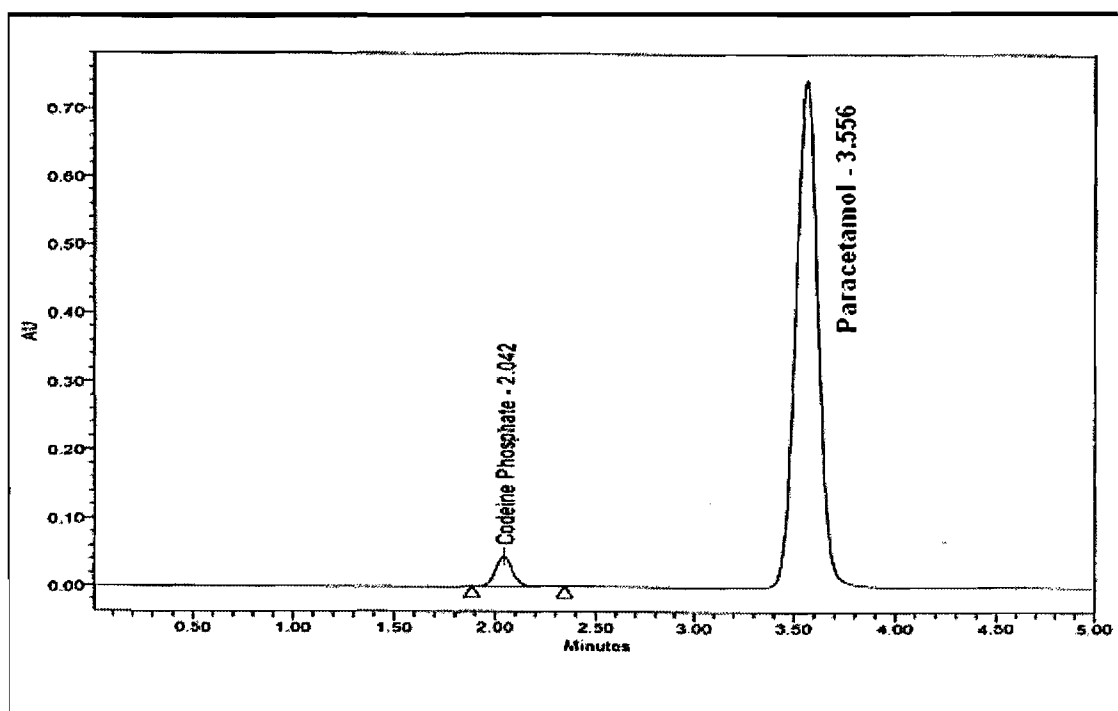


Figure B.2: Standard Chromatogram of codeine phosphate and paracetamol solution

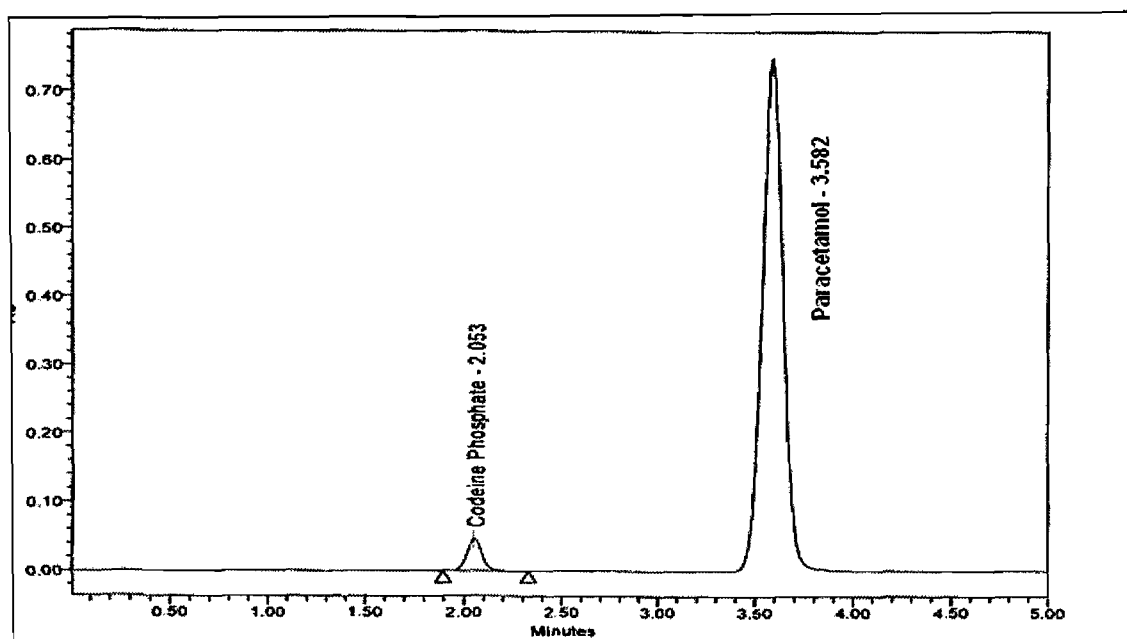


Figure B.3: Sample Chromatogram of codeine phosphate and paracetamol solution

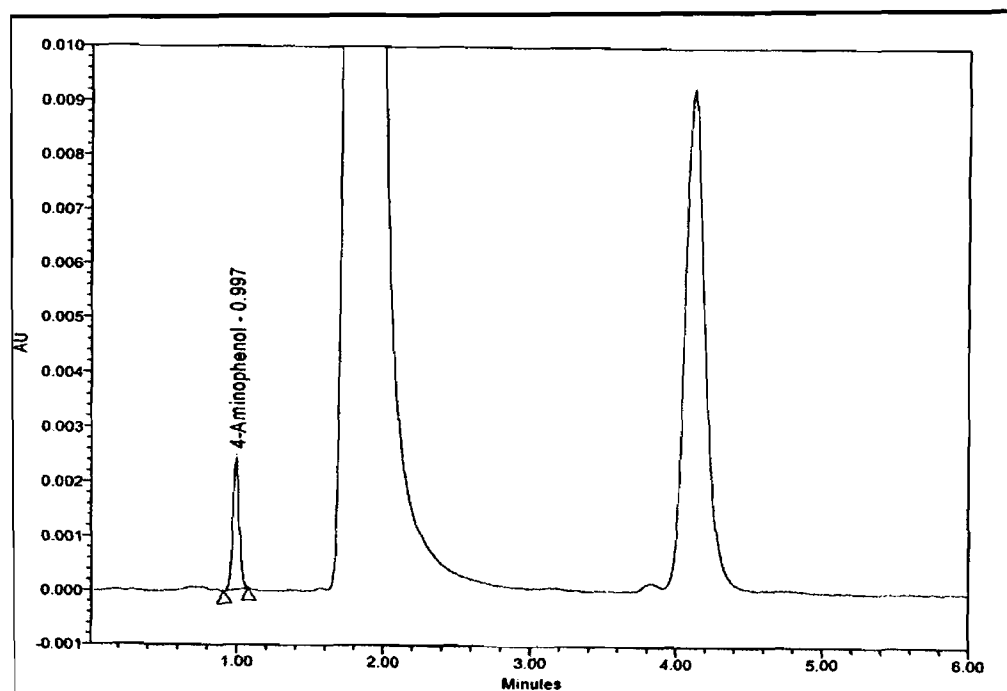


Figure B.4: Standard Chromatogram of 4-aminophenol

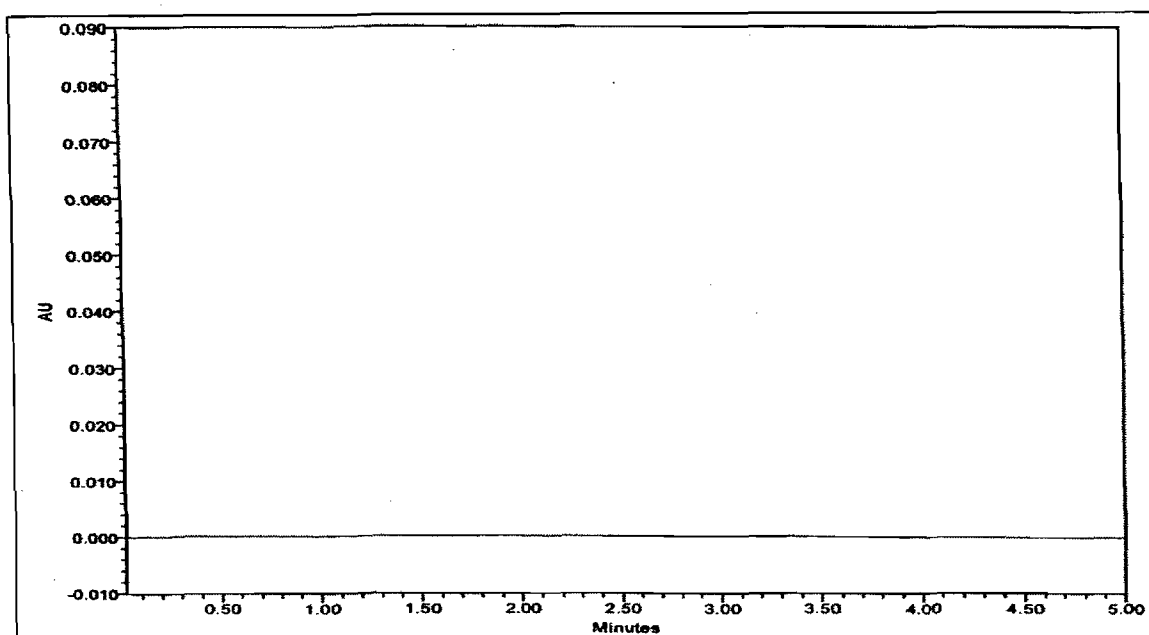


Figure B.5: Placebo Chromatogram at 362 nm

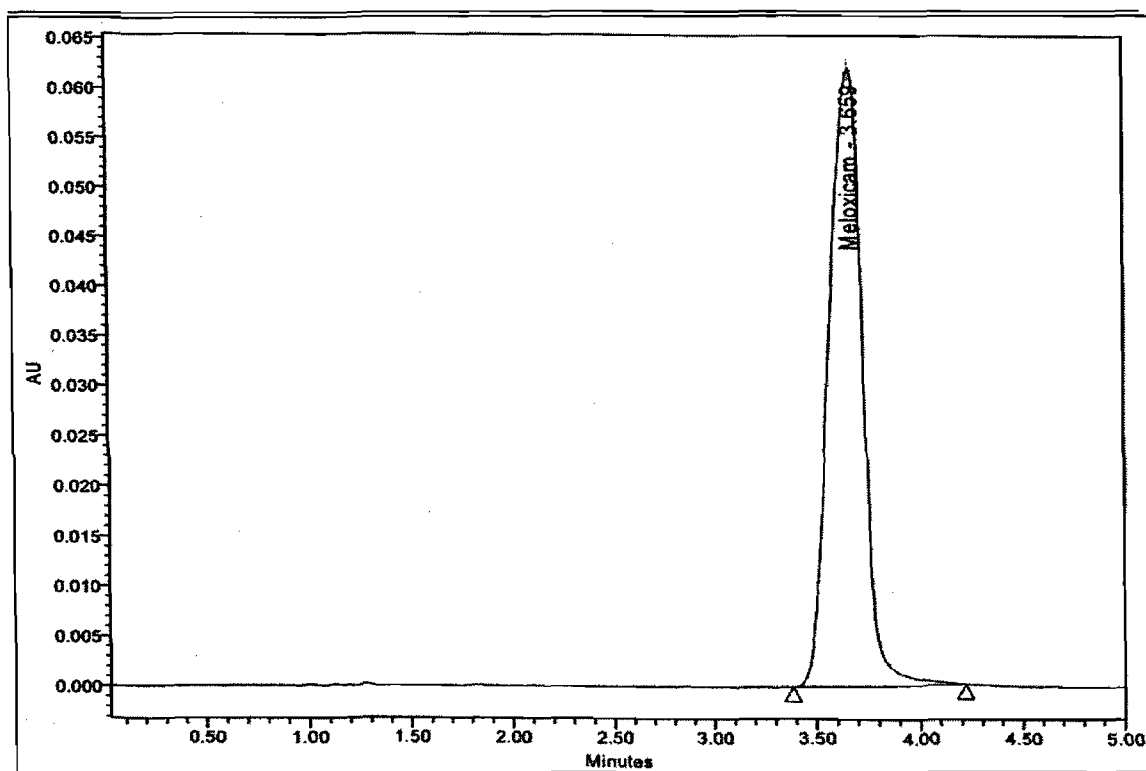


Figure B.6: Standard Chromatogram meloxicam at 362

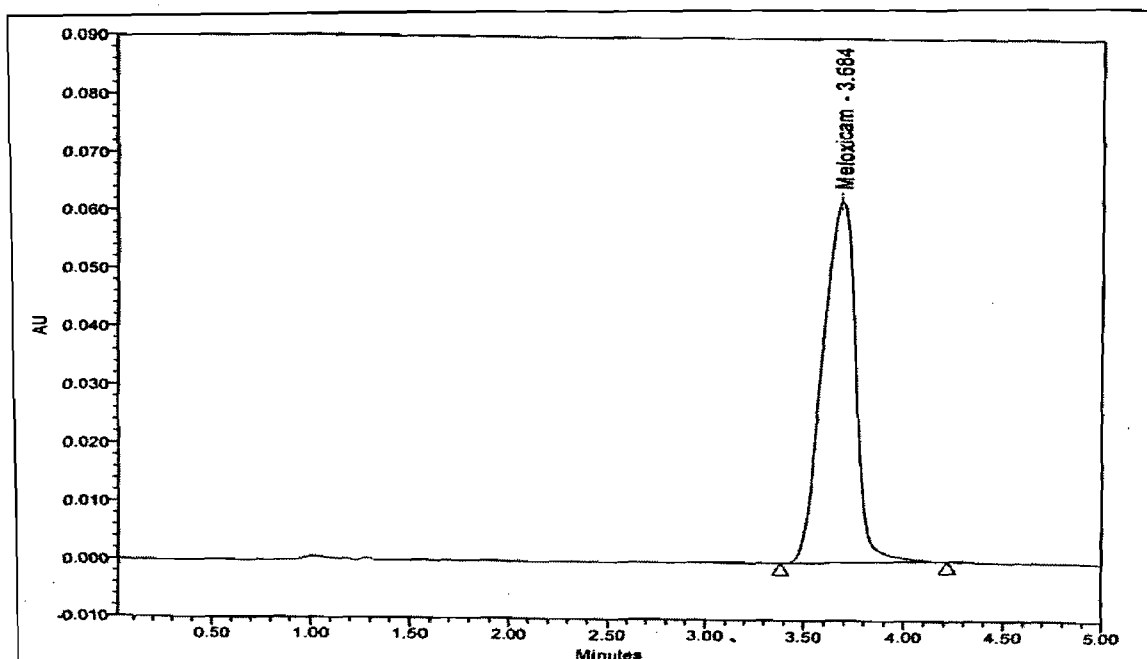


Figure B.7: Sample Chromatogram of meloxicam at 362 nm

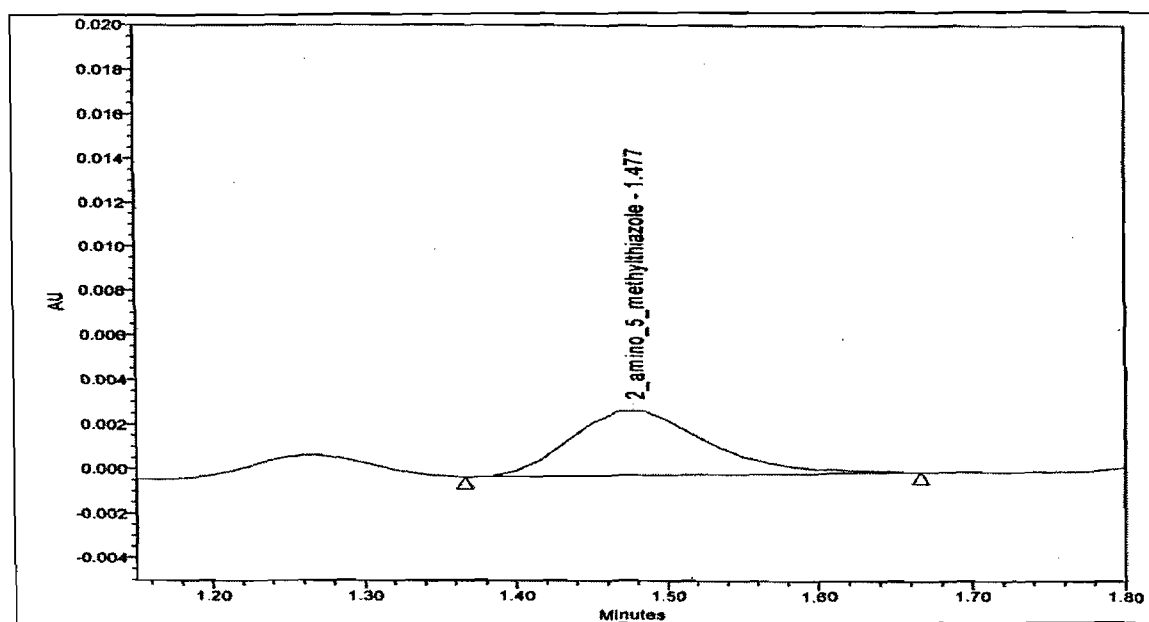


Figure B.8: Standard Chromatogram of 2-amino-5-methylthiazole at 254 nm

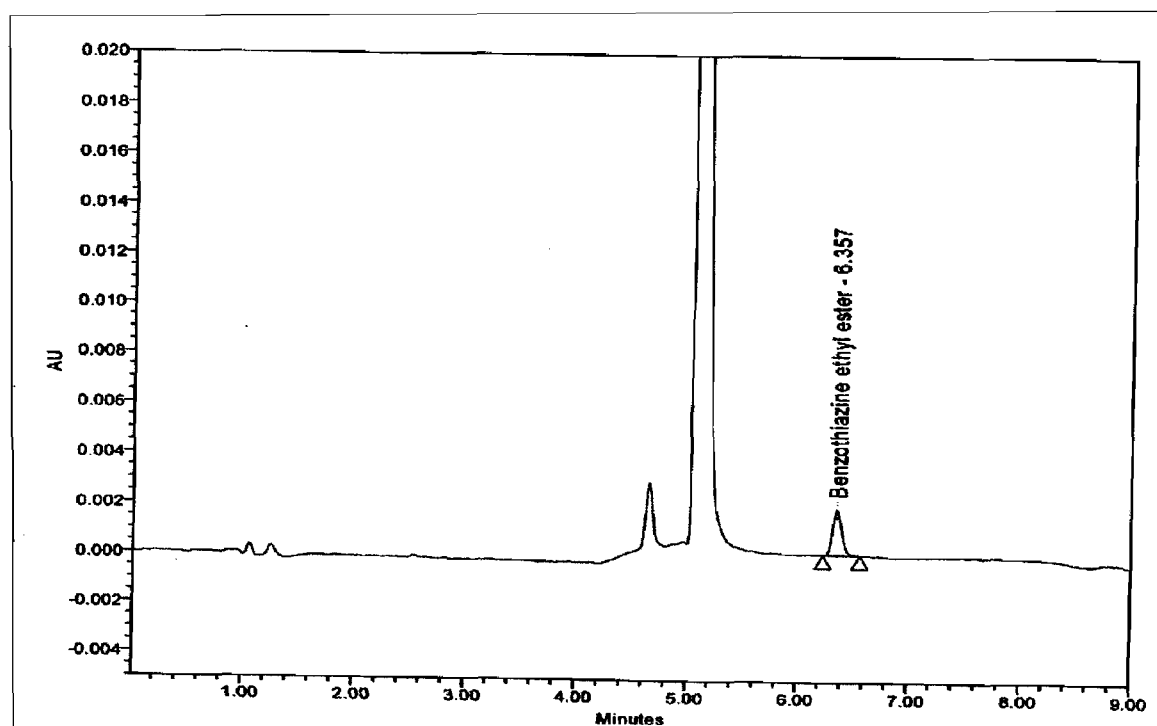


Figure B.9: Standard Chromatogram of benzothiazine ethyl ether at 315 nm

APPENDIX C

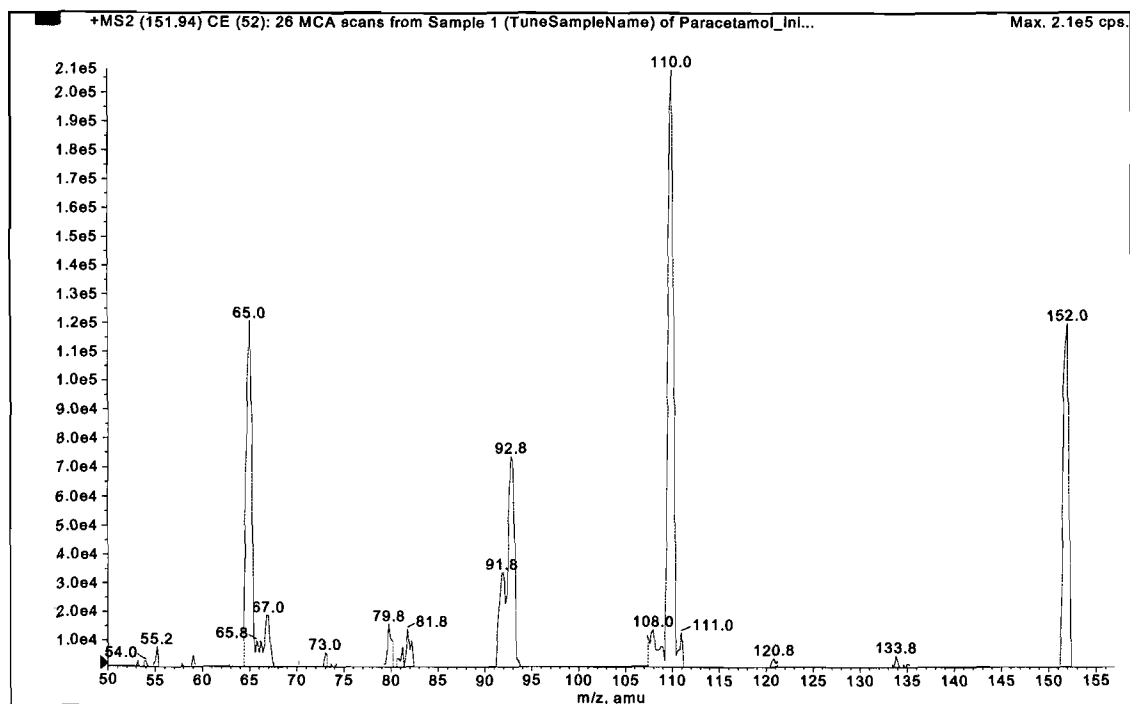


Figure C.1: MS/MS Spectrum of paracetamol

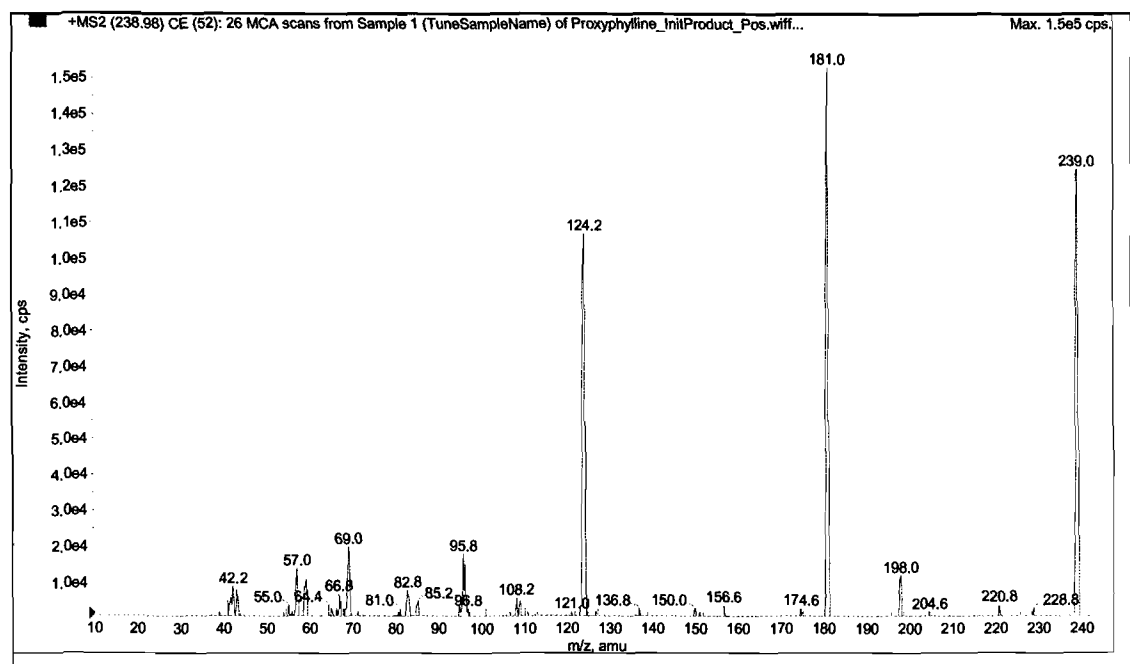


Figure C.2: MS/MS Spectrum of proxyphylline

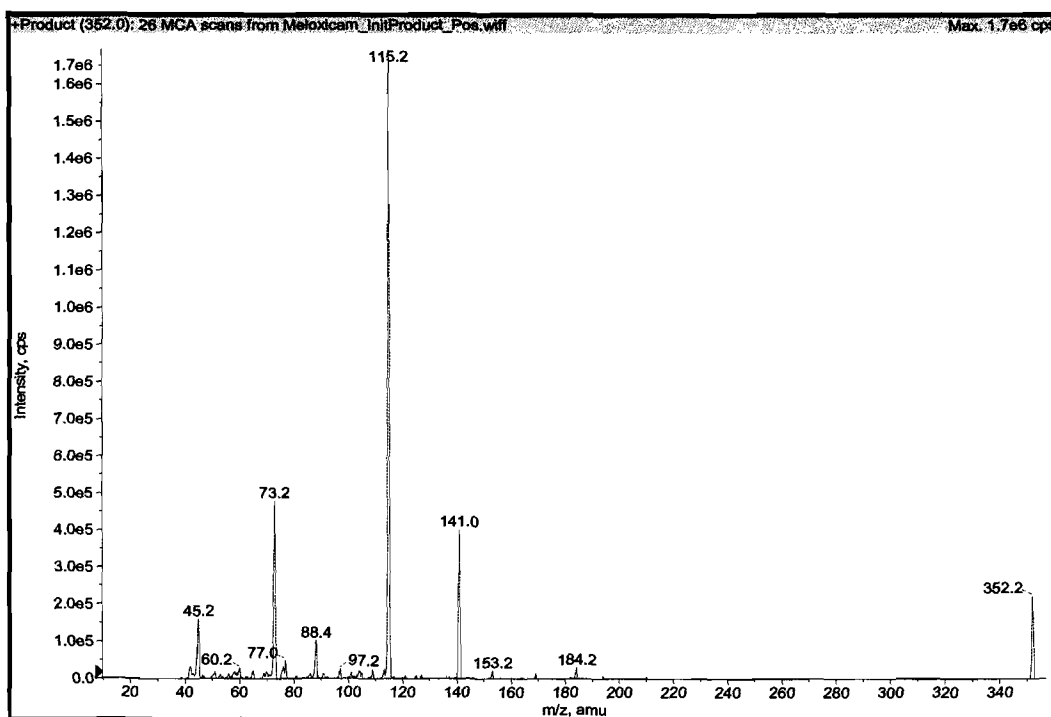


Figure C.3: MS/MS Spectrum of Meloxicam

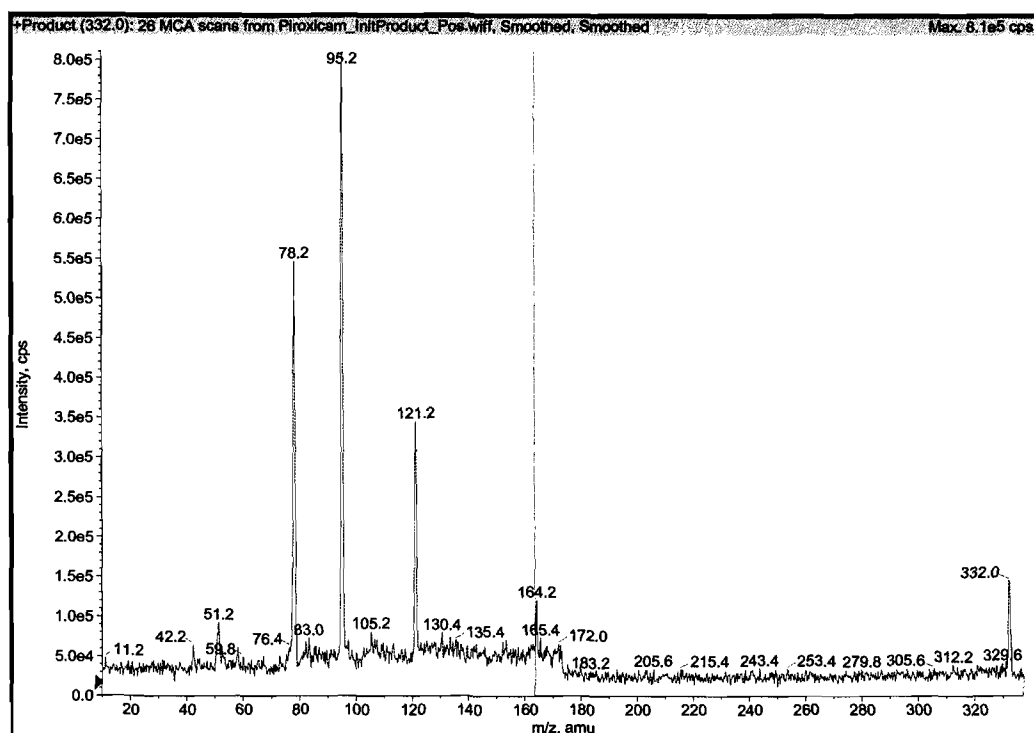


Figure C.4: MS/MS Spectrum of Piroxicam

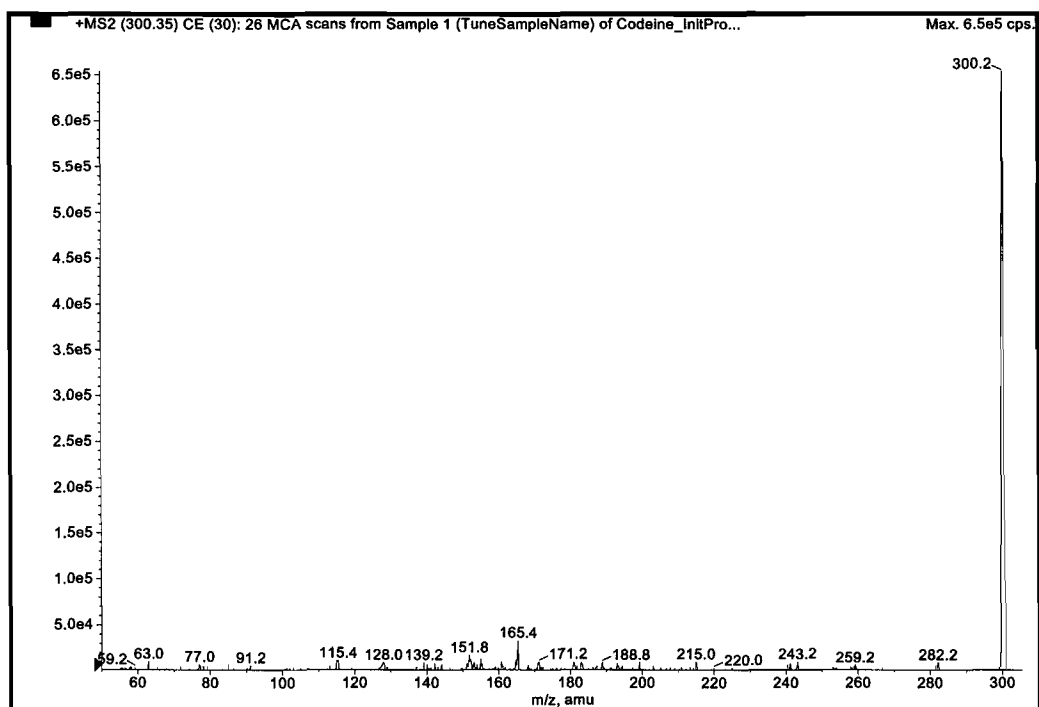


Figure C.5: MS/MS Spectrum of codeine

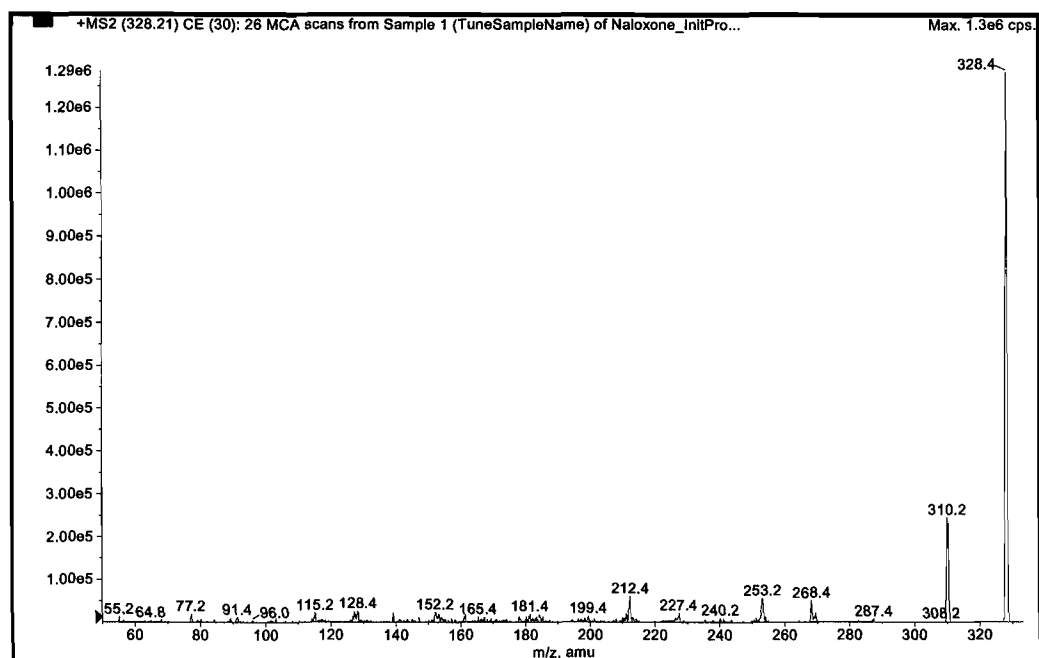


Figure C.6: MS/MS Spectrum of Naloxone

APPENDIX D

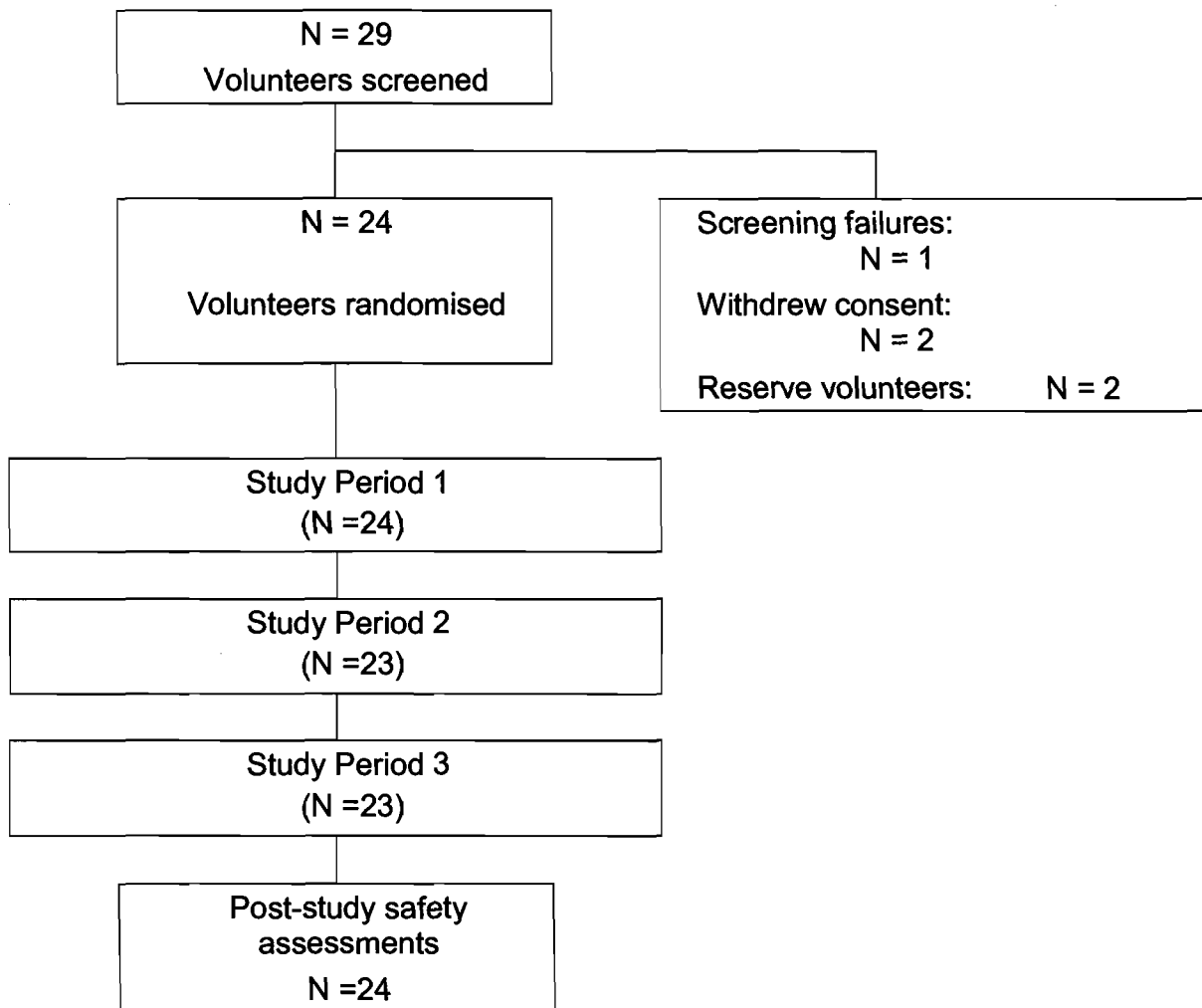


Figure D.1: Volunteer disposition

APPENDIX E

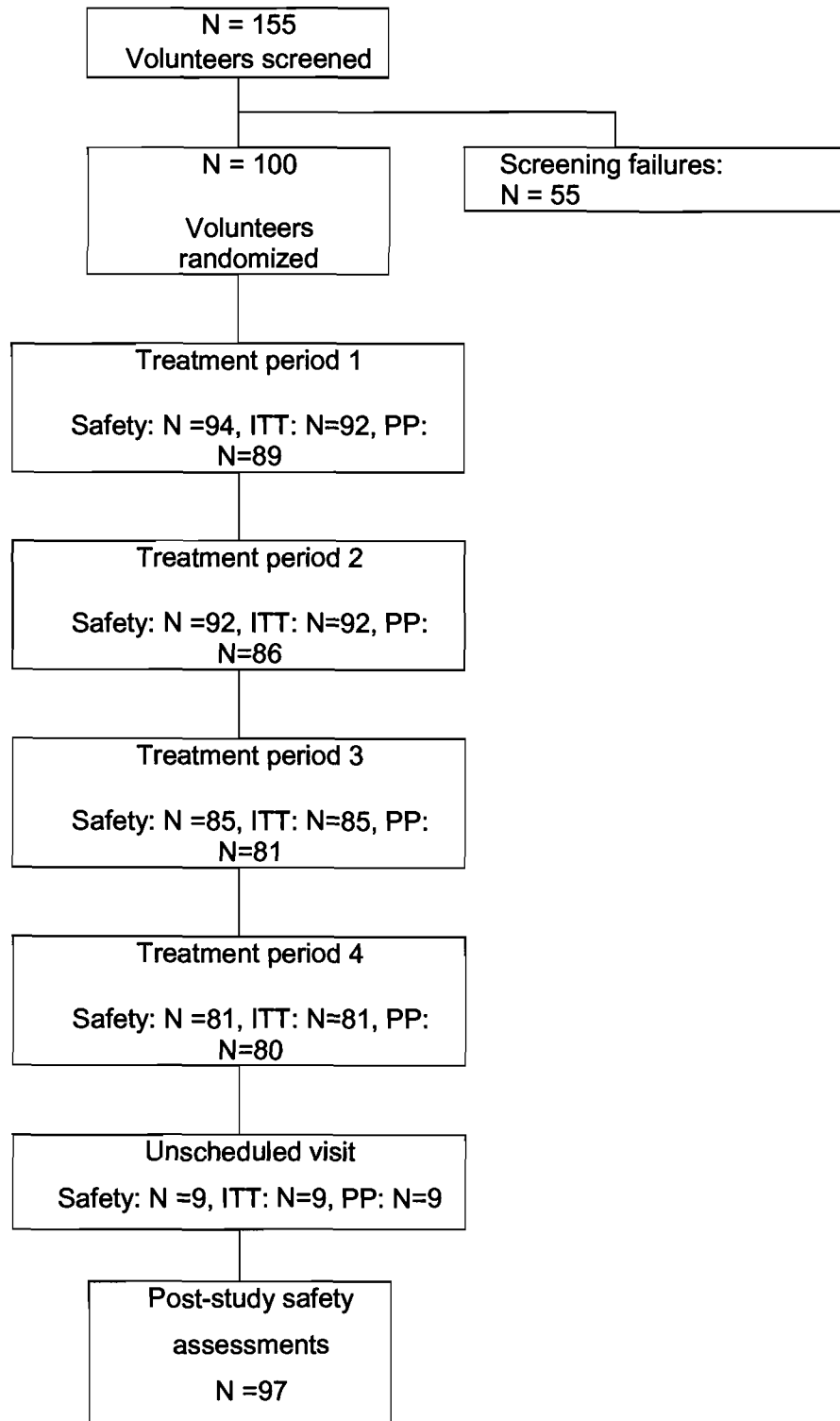


Figure E.1: Volunteer Disposition

SACT 14/2005

**BEFORE TAKING THE FIRST
CAPSULE**

Random.
No.

Initials

DD

MMM

YYYY

Pain intensity rating

HH

MM

***Pain intensity rating: "How much pain are you in now?"**

Rating:

(Rate as follows: 0 = no pain, 1 = mild pain, 2 = moderate pain, 3 = severe pain)

*Note: If pain is moderate to severe, and you are going to take study medication, do a pregnancy test FIRST. Then, if negative, record the time you took the medication in the box below. If the pregnancy test is positive please complete the result below and contact Dr ____ or one of her team members. If the pain is mild or none, complete the pain intensity rating throughout the diary and return the diary to QdotPharma.

First urine pregnancy test

Time of first urine pregnancy test

Result of first urine pregnancy test:

____¹
Positive

____²
Negative

____⁶⁶
Not done

*Please draw a line through the required information for the first pregnancy test should there be any reason why the second urine pregnancy test kit must be used and record the information for the second pregnancy test below. Draw a line through the required information for the second pregnancy test if only the first pregnancy test kit was used.

Second urine pregnancy test

**Time of second urine pregnancy test
(HH:MM):**

Result of second urine pregnancy test:

____¹
Positive

____²
Negative

____⁶⁶
Not done

SACT 14/2005

**BEFORE TAKING THE FIRST
CAPSULE**

Random.
No.

Initials

DD

MMM

YYYY

Dosing

**Please remember to take one capsule out of each securitainer (no.1 and no.2).
Please confirm that you have done so by ticking: —**

Time of 1st dose (HH:MM):

:

SACT 14/2005	_____ HOUR AFTER TAKING THE FIRST CAPSULE					
<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black; text-align: center; padding: 5px;"> Random. No. </td> <td style="width: 50%; border-bottom: 1px solid black; text-align: center; padding: 5px;"> Initials </td> </tr> </table>	Random. No.	Initials	<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 20%; border-bottom: 1px solid black; text-align: center; padding: 5px;">DD</td> <td style="width: 20%; border-bottom: 1px solid black; text-align: center; padding: 5px;">MMM</td> <td style="width: 60%; border-bottom: 1px solid black; text-align: center; padding: 5px;">YYYY</td> </tr> </table>	DD	MMM	YYYY
Random. No.	Initials					
DD	MMM	YYYY				

Pain intensity rating	<table style="margin: 0 auto;"> <tr> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> </tr> <tr> <td colspan="4" style="text-align: center;">HH</td> <td colspan="4" style="text-align: center;">MM</td> </tr> </table>									HH				MM			
HH				MM													
Pain intensity rating: "How much pain are you in now?"																	
(Rate as follows: 0 = no pain, 1 = mild pain, 2 = moderate pain, 3 = severe pain)																	
Rating: _____																	

Pain relief rating	<table style="margin: 0 auto;"> <tr> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> </tr> <tr> <td colspan="4" style="text-align: center;">HH</td> <td colspan="4" style="text-align: center;">MM</td> </tr> </table>									HH				MM			
HH				MM													
Pain relief rating: "How much pain relief have you experienced since taking the first dose of study medication?"																	
(Rate as follows: 0 = none, 1 = a little, 2 = moderate, 3 = a lot, 4 = complete)																	
Rating: _____																	

Rescue medication and Global evaluation of capsule	
Have you needed any paracetamol tablets?	_____ ¹ Yes _____ ²
If YES, record the time of rescue medication (HH:MM): AND fill in the score below:	
Global evaluation of capsule: "How good is this medication in relieving pain compared to what you normally take?"	
(Rate as follows: 1 = poor, 2 = good, 3 = excellent)	
Rating: _____	