An-Najah National University
College of Higher Studies

Comparative Pharmacokinetic Study of Single Oral Doses of Ibuprofen in Healthy Volunteers for Palestinian, British and American Pharmaceutical Equivalents in the local market

By

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Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Environmental Sciences Nablus, Palestine
Aug.16. 2003

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This thesis was defended successfully on Aug.16. 2003 and approved

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Dedication
To My Beloved Ghada and Sons

Acknowledgment

This work has been completed by the support of many individuals whom I would like to express my deep thanks especially to my supervisor Dr. Ansam sawalha for her guidance and continious encouragement, the work team of the chemical analysis and drug quality control center for opening up opportunity to me to carry out analysis of samples and all volunteers for their cooperation. Last but not least, I would express my entire deep thanks to my wife and sons for their patience and consolidation.

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Abstract

This study is conducted so as to test for the existence of actual or potential bioequivalence problems belonging to pharmaceutical equivalents of Ibuprofen drug specifically the Palestinian local quality, British (Nurofen®) and American (Advil®) formulations available in the local market. To carry out this study three samples each of 24 healthy volunteers of both genders (12 males and 12 females) with the following average characteristics: age, weight, height and body mass index 20.667(year), 65.292(kg), 1.6929(m) and 22.750(kg/m^2) respectively were admitted to the central laboratory of An-Najah National university and a single oral dose of 400 mg of each of the formulations was administered. Three blood samples were withdrawn to measure the drugs concentrations at three periods of time (0.5hr, 1hr and 1.5hr following administration). The analysis of blood plasma samples after cleaning them was carried out by utilizing the HPLC/UV. The concentration of Ibuprofen in each sample was calculated and upon analyzing the results statistically, it was verified with a high degree of validation that there is a significant bioequivalence problem regarding the local brand relative to the other pharmaceutical equivalents and the difference between Nurofen® and Advil® was of no significant value.

Chapter one

Introduction

1.1 GENERAL INTRODUCTION:

The introduction of ibuprofen in 1969 was made possible by the labor of many British over 15 years. The search began with over 600 potential molecules, ranging from those similar to existing pain medications to those that were originally designed as weed killers. The path to ibuprofen's discovery is lined with many failures. Several different molecules were used in clinical trials and most produced unwanted side effects, but finally ibuprofen succeeded (Nicholson, 1982).

Since its introduction in 1969, ibuprofen has become one of the most common painkillers and one of the top 200 dispensed drugs in the world. Ibuprofen is an NSAID (non-steroidal anti-inflammatory drug) and, like other drugs of its class, it possesses analgesic, antipyretic and anti-inflammatory properties. Ibuprofen was sought as a safer, more-effective alternative to either corticoids or aspirin for use with rheumatoid arthritis patients. In the course of ibuprofen's discovery in the late 1900's, a link was found between sunburn in guinea pigs, headaches, arthritis, and menstrual cramps (Adams, 1987).

The responsible Ministries of Health are constantly emphasizing the quality, efficacy and safety of pharmaceutical products to safeguard their own public.

With the increasing availability of generic products in the Palestinian market, it is imperative that the Ministry of Health, interested people, and educational institutions introduce a mechanism and conduct studies to further ensure that generic products available are therapeutically equivalent to the innovator's products (an "innovator" product is a medicinal product authorized and marketed on the basis of full dossier i.e. including chemical, biological, pharmaceutical, pharmacological-toxicological and clinical data.) and are clinically interchangeable.

In practice, demonstration of bioequivalence (BE) is generally the most appropriate method of substantiating therapeutic equivalence between medicinal products. Such studies should be conducted according to the international ethical and scientific standards based on good science. The studies should be well designed and comply with Good Clinical & Laboratory Practice (GLP).

To exert an optimal pharmacotherapeutic action an active substance should be delivered at the site of its action in an effective concentration during the desired period.

In this context, Bioavailability (BA) testing of drug products in humans provide the most appropriate method available for determining bioequivalence, where two drug products are considered bioequivalent if they are pharmaceutically equivalent or alternative and their bioavailabilities after administration in the same molar dose are similar to such a degree that their effects can be expected to be essentially the same.

For all the above-mentioned reasons, we decided to conduct a study comparing the local market available ibuprofen pharmaceutical equivalents. This study may provide information & recommendations, which will assist in the formulation of future policy to optimize the bioavailability of current and newly designed therapeutic entities, to encourage Liaisons between academic, industrial, and eligible government laboratories & to integrating information useful for maximizing drug

bioavailability at the drug design stage in order to further protect and safeguard the Palestinian public health.

1.2 Nonsteroidal anti-inflammatory drugs:

The use of the bark from the Willow, utilized over centuries, for the relief of pain and inflammation was described in 1763 by Rev Edmond Stone, and was followed in 1860, with the synthesis of salicylic acid. Aspirin was discovered in 1898 as acetyl salicylic acid (Weissman, 1991).

It was, ultimately, together with its associated compounds, to become the mainstay of therapy for inflammation and the principal pharmacological agents for the management of the rheumatic diseases (Weissman, 1991).

The NSAIDs now constitute perhaps the most frequently prescribed class of medications (Baum, 1985).

They are frequently misused and abused, and are available in multiple formats, including "over the counter " preparations. They can be classified into several groups (Baum, 1985), illustrated in Figure (1).

- **Salicylic acid derivatives** (e.g. Aspirin, sodium salicylate, choline magnesium trislicylate, salsalate, diflunisal, salicylsalicylic acid, sulfasalazine, olsalazine).
- **Para-aminophenol derivatives** (e.g. Acetaminophen)
- **Indole and indende acetic acids** (e.g. Indomethacin, sulindac, etodolac).
- **Heteraryl acetic acids** (e.g. Tolmetin, diclofenac, ketorolac).
- **Arylpropionic acids** (e.g. Ibuprofen, naproxen, flubiprofen, ketoprofen, fenoprofen, oxaprozin).
- **Anthranilic acids** (fenamates) (e.g. Mefenamic acid, meclofenamic acid).
- **Enolic acids** (e.g. Oxicams (piroxican, tenoxicam),
- **Alkanones** (e.g. Nabumetone).

Figure 1: Chemical Classification of Analgesic, Antipyretic, and Nonsteroidal Anti-inflammatory Drugs.

There are relatively few clinical trials comparing the effectiveness of different NSAIDs. These trials have not demonstrated any consistent superiority of one NSAID over another. Differences that have been published can often be explained by the failure to use equivalent doses (March, 1994).

The major risk of NSAIDs is gastrointestinal ulceration associated with bleeding or perforation, both of which can be fatal (Fries, 1993).

This risk is increased with higher doses and longer duration of therapy and is increased in the elderly. The relative risk of this complication has been studied in a practice setting, and differs between the different NSAIDs (Langman, 1994) (Table 1).

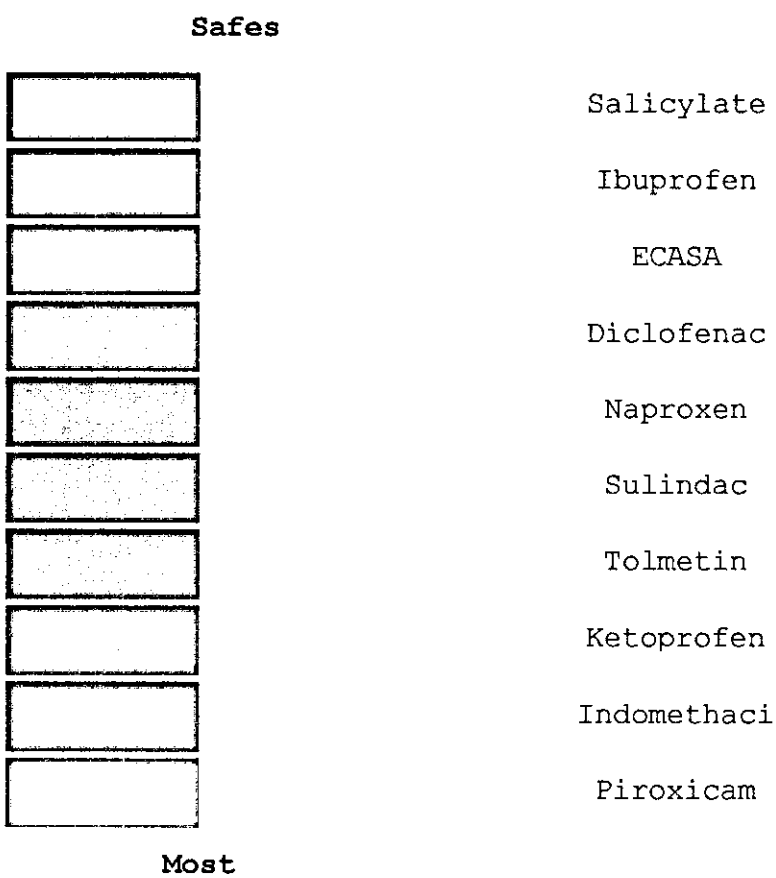


Table 1: Rank According to Risk of Gastrointestinal Toxicity.

Another common adverse consequence is the capacity of NSAIDs to elevate blood pressure; in a recent meta-analysis the magnitude of this effect was estimated to be 5 mm Hg (Johnson, 1994).

In patients on long-term therapy this would lead to a significantly increased risk of hypertension-related morbidity. NSAIDs can also cause salt and water retention and may precipitate congestive heart failure in susceptible patients. They can also inhibit platelet aggregation leading to bleeding (Johnson, 1994).

1.2.1 Efficacy of NSAIDs:

A considerable number of NSAIDs with proven efficacy and safety are currently available for prescription and nonprescription use. Many patients with acute pain obtain good pain relief with these drugs. Pharmacotherapy may be an important component of a multimodal or balanced approach to the management of pain (Ashburn, 1999) "Drug therapy is the mainstay of treatment for the management of acute pain," according to the American Pain Society. Three facts relative to the efficacy of non-opioid analgesics for acute pain management need to be

Society. Three facts relative to the efficacy of non-opioid analgesics for acute pain management need to be mentioned: (1) NSAIDs possess proven efficacy for mild to moderate acute pain. (2) The potency of some NSAIDs is greater than that of others. Several of the newer NSAIDs have demonstrated potency comparable to or greater than that of opioid agents and are indicated for postoperative pain management (American Pain Society, 1992; Zuckerman, 1998; Brooks, 1991). (3) Since individual patient response varies considerably, therapeutic failure with one agent does not preclude success with another. Analgesics may require adjustment until the patient reports adequate pain relief. Such an adjustment can be guided by the "analgesic ladder" promulgated by the Cancer Pain Relief and Palliative Care Program of the World Health Organization.

1.2.2 Mechanism of action:

The main mechanism of action of NSAIDs was clarified by Sir John Vane in 1971. He noted the inhibition by aspirin, of prostaglandin synthesis (Vane, 1971).

It was noted that cells synthesized prostaglandins in response to tissue injury, and inhibition of these prostaglandins inhibited inflammation. Whilst the prostaglandin hypothesis is generally accepted as being the most important, we now know that the mechanisms are more complex and involve additional non-prostaglandin dependent pathways (Vane, 1971). In the prostaglandin-mediated process, cyclooxygenase metabolizes arachidonic acid to form prostaglandin endoperoxides including prostaglandins, prostacycline and thromboxane.

Blocking of the cyclooxygenase enzyme results in a reduction in the formation of prostaglandins. The prostaglandins in fact are both physiologically important and potentially pathologically harmful. PGE_2 is the principal eicosanoid formed from arachidonic acid. It is a crucial mediator of inflammatory changes (Portanova, 1996).

1.2.2.1 Peripheral Mechanism:

With cellular damage, there is release of products of both the cyclooxygenase and lipoxygenase pathways resulting in prostaglandins and leukotrienes where Prostaglandins sensitize afference neurons (nociceptors) to noxious stimuli such as chemical, heat, and mechanical pressure (Chahl, 1977; Birrell, 1991).

Prostaglandins and leukotrienes probably do not activate the nociceptors directly, but work through activation of cyclic adenosine monophosphate (CAMP), and/or activation of products of polymorphonuclear cells. Inhibition of prostaglandins or leukotrienes by NSAIDs results in analgesia (Taiwo, 1991; Martin, 1990).

1.2.2.2 Central Mechanism:

Evidence now indicates that NSAIDs are antihyperalgesic through a direct action on the spinal cord (central mechanism) (Malmberg, 1992). This effect can be dissociated from the peripheral action of NSAIDs and may be dissociated from their anti-inflammatory mechanism.

As a painful stimulus activates the peripheral nociceptor, stimuli are sent along the afferent C fibers into the dorsal horn where they synapse with second-order neurons for transmission to higher centers. One of these mechanisms, known as windup, allows for amplification of an incoming signal in both intensity and duration to create a state of hyperalgesia. With repetitive C-fiber nociceptor stimulation from the periphery, excitatory amino acids (neurotransmitters) such as glutamate and aspartate, as well as several peptides (including substance P) are thought to increase. These cause activation of NMDA (N-methyl D-aspartate) receptors of the postsynaptic second neuron in the dorsal horn.

Activation of these receptors is not thought to function to transmit nociceptive information evoked by a peripheral stimulus, but rather to facilitate the processing of such incoming signals to create a state of hyperalgesia (windup in the dorsal horn). Experiments utilizing intrathecal NSAIDs in the rat model (Malmberg, 1992), as well as antagonists to the NMDA receptor, suppressed the hyperalgesic component but not the acute pain behavior of the pain stimulus.

In addition, intrathecal injection of several cyclooxygenase products such as PGE_2 and PGD_2 also functioned in this model to induce a hyperalgesic state. NSAIDs are thought to block activation of the NMDA receptor induced by excitatory amino acids released by repetitive C-nociceptor firing. This is thought to occur through calcium-induced release of prostaglandins in the dorsal horn. By blocking this activation in the experimental model, NSAIDs are thought to be antihyperalgesic. Lastly, NSAIDs block windup at doses much lower than doses required for systemic effects.

Thus, NSAIDs work in the periphery by decreasing the sensitivity of the nociceptor to painful stimuli induced by heat, trauma, or inflammation. In the central nervous system, they are thought to function as antihyperalgesics and block the increased transmission of repetitive incoming signals to higher centers.

In effect, they modulate perception of pain (which is enhanced through windup in the dorsal horn) caused by repetitive stimulation from the periphery. Since they function by modulation of the perception of pain, they may

be useful when given in the preoperative period and may reduce the need for postoperative analgesia. The explosion of knowledge about the different cyclooxygenase enzymes has given us a more fundamental understanding of the actions and side effects of these drugs.

In the 1980's Needleman showed that COX enzyme was increased in inflamed tissue and that COX was stimulated by interleukin-1 (IL-1) on cultured human dermal fibroblasts. They showed a dose-dependent response curve. This suggested IL-1 dependent transcriptional regulation (Raz, 1988).

In 1990 he demonstrated the induction of COX by endotoxin. An increase in COX was prevented by glucocorticoids. However it was noted that Dexamethasone did not affect baseline prostaglandin formation.

They therefore postulated a second COX enzyme. In 1991 the second COX isoform was cloned. This represented what is now known as COX-2. COX-1 is now known to be present in most tissues as the housekeeper enzyme. COX-2 is inducible by inflammation. It is not present at baseline,

but increases in response to inflammation including arthritis. It has 60% homology with COX-1.

Both have the same affinity to convert arachidonic acid to prostaglandin. COX-1 maintains normal gastric mucosa and influences kidney function. The inhibition of COX-1 is therefore undesirable. The inhibition of COX-2 on the other-hand is a desirable effect figure (3)

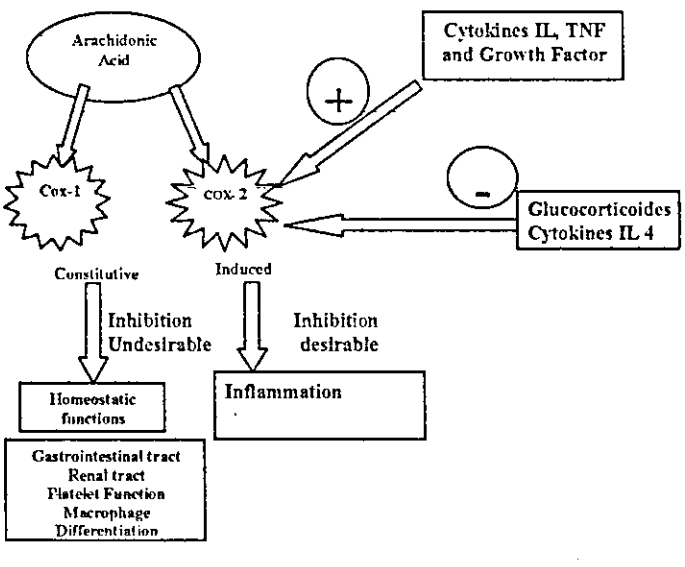


Figure 3: the current COX concept.

The concept of the COX-1 to COX-2 ratio provides us with a mechanism to assess the balance of inhibition of the inducible COX-2. Analysis of these ratios and side effects of the older conventional non-steroidal anti-inflammatories show that the higher the ratio, the lower the COX-1 inhibition, and the lower the overall side effect profile see

table (2) bellow for some selected NSAIDs (Engelhardt, 1995).

Compound	COX-1/COX-2
Celecoxib	160
Rofecoxib	77
Acetylsalicylic	0.28
Ibuprofen	0.13
Ketoprofen	0.05

Table (2): Selected COX-1/ COX-2 Ratios for humans.

1.3 Ibuprofen:

1.3.1 Physicochemical properties:

Ibuprofen [(+/-) 2-(4-isobutylphenyl) propionic acid, is one of the most commonly used anti-inflammatory agents. It is a white powder with a melting point of 74-77° C and is very slightly soluble in water (<1 mg/mL) and readily soluble in organic solvents such as ethanol and acetone. It is available in 400, 600 and 800 mg tablets for oral administration. Ibuprofen is considered to be the prototype for the family of synthetic 2-arylpropionic acids, profens Figure (4), is a sub-class of the NSAIDs. The profens have an asymmetric carbon canter attached to a carboxylic acid, a

methyl, and an aryl group of varying structure. Some of the available profen drugs are depicted in Figure (4): ibuprofen (1.1), naproxen (1.2), ketoprofen (1.3), and flurbiprofen (1.4). Ibuprofen is distributed over the counter and naproxen belongs to the top-ten of drugs marketed worldwide in 1989 (Nerurkar, 1992).

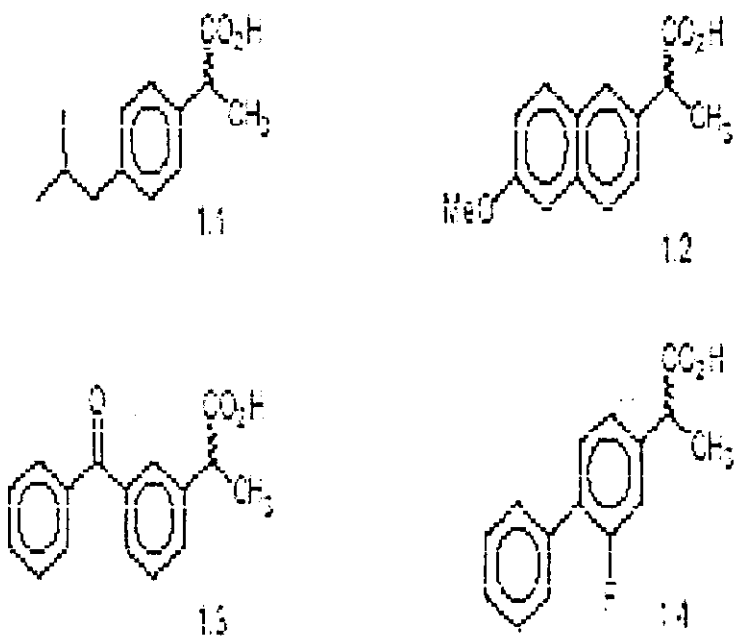


Figure 4 : some of the available profen drugs

1.3.2 In vivo behavior of Ibuprofen enantiomers:

The enantiomers of Ibuprofen differ substantially in both their pharmacodynamics and pharmacokinetic properties figure (5) (Adams, 1976).

It is generally recognized that the S-Ibuprofen is the enantiomer that inhibits prostaglandin synthetase. The absolute configuration, as well as the conformation of this isomer is important for the interactions with the cell receptors responsible for the therapeutic anti-inflammatory activity (Campbell, 1990).

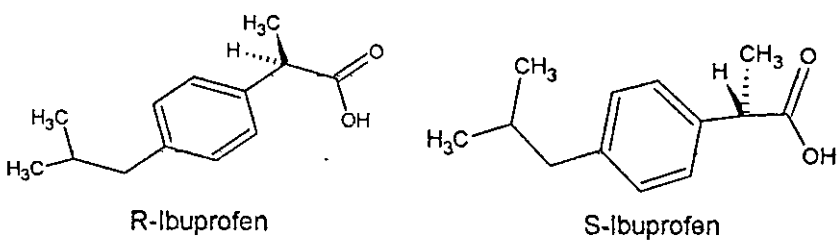


Figure 5: The enantiomers of Ibuprofen.

Prior to the early nineties, the S-enantiomer was regarded as the eutomer (the biological active enantiomer) of Ibuprofen and the R form as the distomer (the biological inactive enantiomer) (Bye, 1990).

Observations in the late eighties made this dissimilarity less clear. In practice, Ibuprofen is generally administered as racemic mixture. In vivo, however, some of it can undergo, to a certain extent, a unidirectional inversion from the R to the S-form, leading to an enantiomeric excess of the S form when aracemate of the drug is administered. This unique

process was supposed to enhance the effectiveness of Ibuprofen racemate as chiral drug. The most compelling mechanism for this inversion figure (6), originally proposed by Nakamura *et al.*, (Nakamura, 1981) is a three-step process which commences with the enantiospecific enzymatic formation of a thioester between the R-enantiomer of the 2-arylpropionic acid and coenzyme A (CoA).

This thioester may be hydrolysed to regenerate the R-enantiomer or may undergo epimerization to yield the thioester in which the 2-arylpropionyl moiety has the S-configuration. Subsequent hydrolysis of this (S)-CoA thioester completes the inversion process. The epimerization step may proceed non-enzymatically, due to the acidic nature of the proton connected to the alpha carbon of the 2-arylpropionic acid substituent of the thioester. This process is clinically important because it generates an active cyclo-oxygenase inhibitor (S-Ibuprofen) from a relatively inactive precursor (R-Ibuprofen). The extent of this dynamic resolution, however, varies in vivo within and between individuals and is certainly not complete (Rudy, 1991; Lee, 1985).

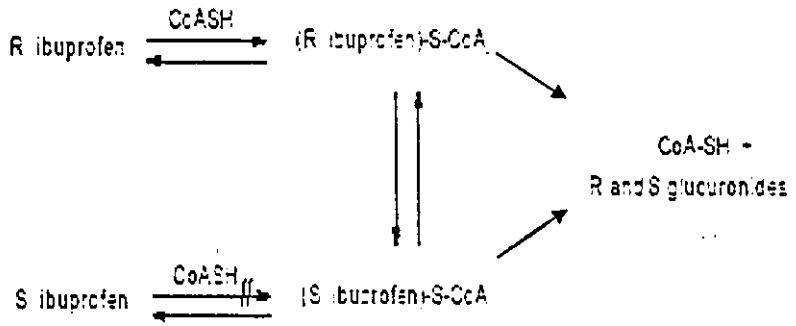


Figure 6: Resolution mechanism of Ibuprofen racemate.

Moreover, this metabolic inversion occurs some time after administration in the elimination phase, when the plasma concentration of the initial S-Ibuprofen is lowered markedly, and the therapeutic effect has dropped considerably.

Hence, the delayed S-Ibuprofen delivery may not contribute to the therapeutic effect. This inversion phenomenon cannot be seen as a "prodrug principle" (Loew, 1989) and leads to great uncertainty in the actual dose of the active S-form of Ibuprofen.

The described metabolic pathway of the R enantiomer can have pharmacological and toxicological effects related to inhibition of prostaglandin synthesis. The ability of the R-enantiomer to form potentially reactive acylglucuronides, to

form thioesters with coenzyme A, to be incorporated into triacylglycerols, and possibly to interfere with lipid metabolism and a host of biological membrane processes, makes the R- enantiomer a pharmacological uncertainty, besides their non-inhibiting property of prostaglandin synthesis.

1.3.3. The importance of the S enantiomer:

The advantages in using the pure S enantiomer of a chiral arylalkanoic acid NSAID, however, comprise the following:

1. The recipient would be exposed to less of the xenobiotic and therefore a reduced metabolic and renal load.

2. Adverse effects, which may be mediated by the R-enantiomer or its metabolites, would be avoided.

3.Enantiomer-enantiomer pharmacokinetic interactions (which may lead to non-linearity in the pharmacokinetics of the active enantiomer) would be avoided.

4. Relationships between drug concentrations in plasma or synovial fluid and therapeutic response would be easier to assess; enantioselective methods would not be

required to measure total and unbound drug concentrations (Evans, 1992).

5. In vivo, the pure enantiomers of 2-arylpropionic acids are absorbed faster than the corresponding racemic compound due to the rate of conversion of the R-isomer to the S-isomer, racemic Ibuprofen can take as long as 40 minutes to take effect, while the same dosage of enantiomerically pure S-ibuprofen takes less than a third of that time.

At high dosages of racemic drugs, concern regarding toxicity of the inactive isomer is high. Therefore, current research with Ibuprofen and other racemic drugs focuses on efficient methods to isolate only the helpful isomer (Sen, 1996; Stock, 1991).

6. Twice the dose of racemic Ibuprofen is needed to attain the same plasma concentration of S form, as compared with the pure S enantiomer (Brown, 1992).

1.3.4. The importance of the R enantiomer:

Some observations in the late eighties revealed that part of the analgesic effects of NSAIDs are not completely explained by the prostaglandin synthesis inhibition effect of

the S-Ibuprofen in inflamed tissue. This analgesic effect is attributed to the R- Ibuprofen. It is presumed to originate from the fact that the R form crosses the blood-brain barrier more easily than the S form, and possibly acts in the spinal cord or central nervous system (CNS) (Jurna, 1990).

1.3.5. The use of single R or S Ibuprofen as drugs: an evaluation:

It can be shown that a selective use of either the pure R or S enantiomers of the profens may be applicable: 1) the S form for curbing inflammation and pain in intensive inflammation, and 2) it may suffice to use pure R profens for suppressing simple pains, particularly by using those profens that do not encounter chiral inversion in man (Brune, 1992).

The reason for continued use of racemic NSAIDs today are probably chiefly economic. In conclusion, the profen drugs should be used as the pure, single enantiomers. More effective drug treatment could be established by using the required pure enantiomer for the specific medical condition.

1.4 Synthesis:

There have been many commercial and laboratory publications for the synthesis of Ibuprofen.

Two of the most popular ways to obtain Ibuprofen are the Boot process and the Hoechst process.

Most of these routes to Ibuprofen begin with isobutyl benzene and use Friedel-Crafts acylation (Tlchm, 1995).

The Boot process requires five steps, while the Hoechst process, with the assistance of catalysts, is completed in only three steps figure (7).

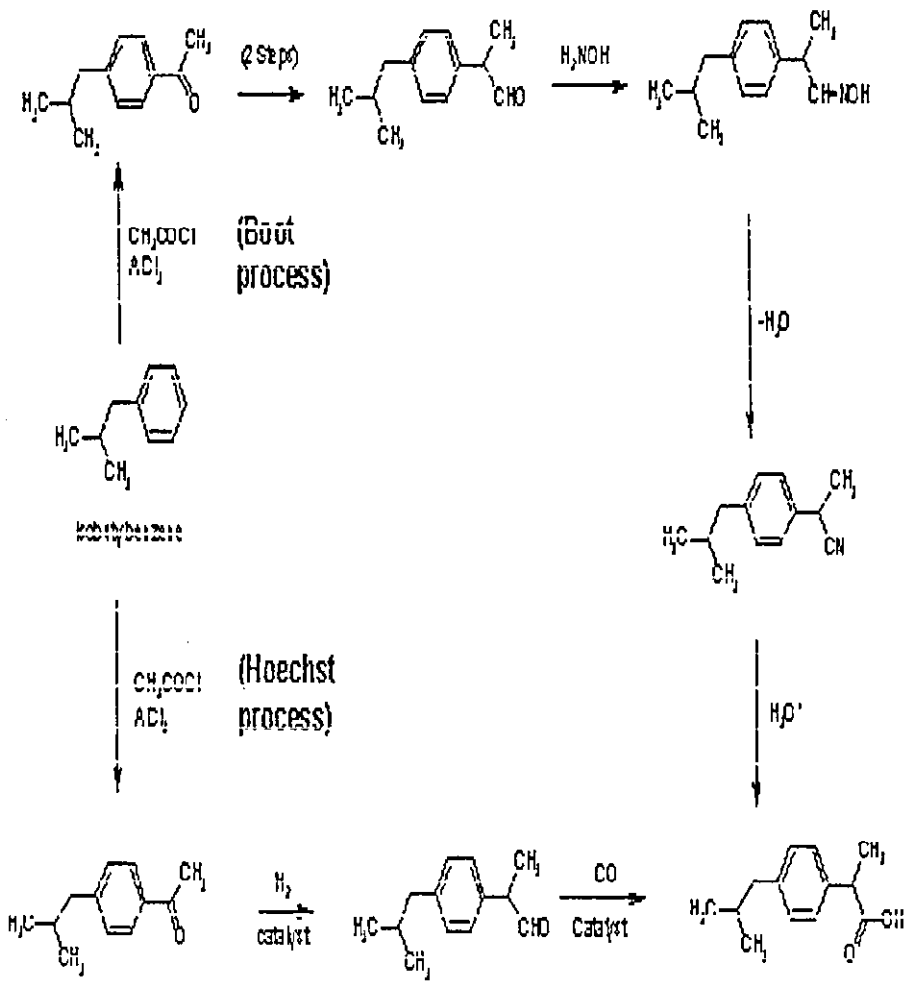
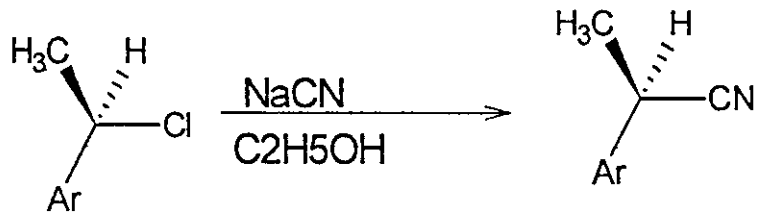


Figure 7: commercial synthesis of Ibuprofen.

Another key step in the synthesis of Ibuprofen in factory is the reaction of chloroalkane A with cyanide ion figures (8,9), but haloalkanes, such as compound A, are considered environmental hazards if spilled. So to avoid this problem, and at the same time make Ibuprofen synthesis faster (Ucla.edu, 1995), an analog of haloalkane A that does not have a halogen atom but will undergo a faster S_N2 reaction than A is seen in figure (10).



(A)

Figure 8: reaction of chloroalkane A with cyanide ion.

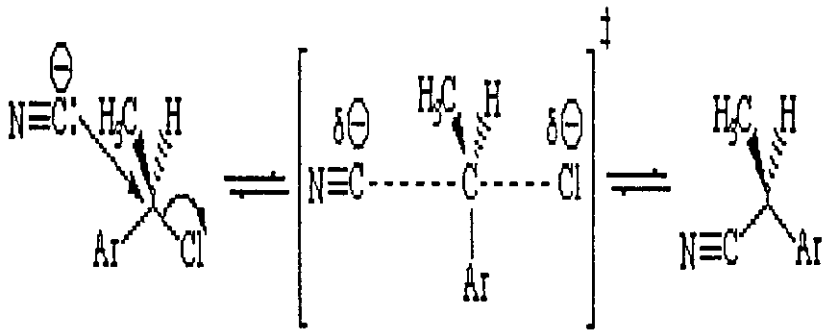


Figure 9: S_N2 reaction of cyanide with chloroalkane.

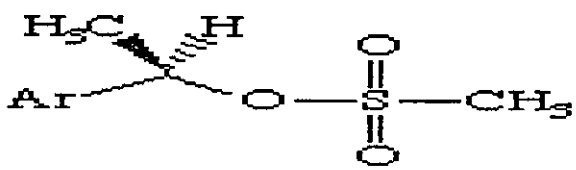


Figure10: ananalogofhaloalkane

The last step in the Ibuprofen synthesis is hydrolysis of the nitrile group to a carboxylic acid see figure (11)

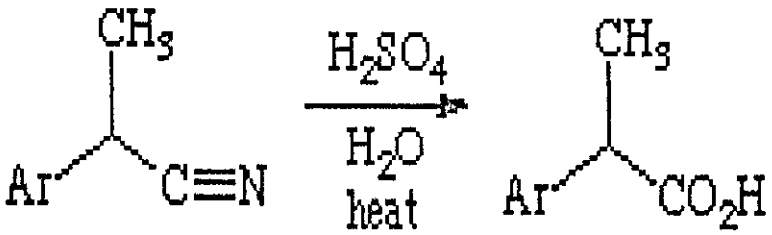


Figure 11: hydrolysis of the nitrile group to a carboxylic acid

1.4.1 Potential environmental adverse effects of NSAIDs residues:

The active ingredients of pharmaceuticals that are diverse in structure, chemical and biochemical activity are used in large quantities throughout the world and their production some times needs raw materials that have real environmental adverse effects like Carbon tetrachloride (Ozone destroying solvent) which is consumed and emitted in the Shasun Ibuprofen process . These compounds are continually discharged into all environmental compartments via industrial and domestic effluents.

Reports of detection of intact pharmaceuticals and their metabolites in effluents of wastewater treatment plants, and in surface waters and groundwater in Europe and the U.S.A are on the increase Table (3) bellow (Buser, 1999).

Compound	Waste water treatment plant		Lake	
	influent	effluent	influent	effluent
Clofibric acid	= 100	= 100	2 - 11	2 - 9
Diclofenac	470 - 1920	310 - 930	11 - 270	< 1 - 12
Ibuprofen	990 - 3300	2 - 81	< 0.2 - 2	0.2-0.8

Table (3): Concentrations of clofibric acid, diclofenac, and ibuprofen detected in samples from the catchment area of Greifensee Swiss Lake (ng/L).

However, very little is known about the environmental behavior and fate of pharmaceutical compounds and about their effects on aquatic ecosystems. In a preliminary investigation that has been performed to examine the environmental effects of a mixture of three pharmaceuticals: Ibuprofen, ciprofloxacin, and fluoxetine. Concentrations of the ternary mixture were (ibuprofen, ciprofloxacin, fluoxetine): Control (0, 0, 0); low treatment (LT: 6 g/L, 10 g/L, 10 g/L); medium treatment (MT: 60 g/L,

100 g/L, 100 g/L); and high treatment (HT: 600 g/L, 1000 g/L, 1000 g/L). This pilot microcosm study demonstrated impacts at multiple trophic levels including aquatic macrophytes and fish. Water milfoil (Myriophyllum sp.) showed severe growth inhibition in the HT groups as compared to controls. Duckweed (Lemna gibba) growth was halted and necrosis predominated in the MT and HT groups while LT showed increased growth compared to controls. Sunfish (Lepomis gibbosus) showed 100% acute (96 hr) mortality in the HT group and ~46.6% mortality after 35 days in the MT group. (D. J. Johnson, 1995).

In the same direction and toward decontamination trials of different environmental compartments, phytoremediation of high-volume pharmaceuticals in aquatic plant systems was suggested, so studies on the response of plants to pharmaceutical exposure in aquatic phytoremediation systems were coducted. Several high-volume pharmaceuticals or their common metabolites were investigated: acetaminophen, ibuprofen, salicylic acid and clofibric acid. The aquatic macrophyte *Myriophyllum aquaticum* was exposed separately to each of these pharmaceutical compounds for a period of 7-14 days at an

initial concentration of 8-10 mg/L. At this exposure level, acetaminophen and salicylic acid disappeared rapidly, while ibuprofen and clofibric acid proved recalcitrant (Umasssoils, 2001).

1.5 Uses:

Ibuprofen is used to relief the symptoms of a wide range of illnesses such as headaches, backache, period pain, dental pain, neuralgia, rheumatic pain, muscular pain, migraine, cold and arthritis. Recently evidence has emerged suggesting that ibuprofen is effective in the treatment of Alzheimer's disease. To stop Alzheimer's disease, a leading theory proposes reducing in-brain buildup of a protein fragment known as beta-amyloid. Scientists struggling to achieve this goal are, for example, testing a vaccine that prompts the immune system to clear this amyloid from the brain (Lim, 2000).

The solution may be even simpler. A study in mice suggests that ibuprofen, the common nonprescription drug, may lessen abnormal accumulation of beta-amyloid.

About 20 studies have revealed that people who took NSAIDs for various reasons had a smaller risk—60 percent

less in one study—of developing Alzheimer's disease than people who didn't take the drugs (Veld, 2001).

Effects of NSAIDs such as aspirin and ibuprofen on lung cancer risk were assessed also by estimating odds ratios (relative risks) with 95% confidence intervals and performing trend tests. Daily intake of NSAIDs for at least 2 years prior to interview was associated with a 68% reduction in the relative risk of lung cancer (RR, 0.32; 95% CI, 0.23-0.44; $p<0.01$). The inverse trend of lung cancer risk with increasing NSAID use was highly significant ($p<0.01$). Results were similar for men (RR, 0.41) and women (RR, 0.22), and for the individual compounds, aspirin (RR, 0.25) and ibuprofen (RR, 0.39). These results combined with the current molecular evidence suggest that regular NSAID intake may prevent tobacco carcinogenesis through COX-2 blockade (Randall, 2002).

1.6 Adverse effects:

1.6.1 Risk of GI Ulceration, Bleeding and Perforation with NSAID Therapy:

Serious gastrointestinal toxicity such as bleeding, ulceration, and perforation can occur at any time, with or

without warning symptoms, in patients treated chronically with NSAID therapy. Although minor upper gastrointestinal problems, such as dyspepsia, are common, usually developing early in therapy, physicians should remain alert for ulceration and bleeding in patients treated chronically with NSAIDs even in the absence of previous GI tract symptoms.

In patients observed in clinical trials of several months to two years duration, symptomatic upper GI ulcers, gross bleeding or perforation appear to occur in approximately 1% of patients treated for 3-6 months, and in about 2-4% of patients treated for one year. Physicians should inform patients about the signs and/or symptoms of serious GI toxicity and what steps to take if they occur (druginfonet, 2001).

Studies to date have not identified any subset of patients not at risk of developing peptic ulceration and bleeding. Except for a prior history of serious GI events and other risk factors known to be associated with peptic ulcer disease, such as alcoholism, smoking, etc., no risk factors (e.g., age, sex) have been associated with increased risk.

Elderly or debilitated patients seem to tolerate ulceration or bleeding less well than other individuals and most spontaneous reports of fatal GI events are in this population. Studies to date are inconclusive concerning the relative risk of various NSAIDs in causing such reactions.

High doses of any NSAID probably carry a greater risk of these reactions, although controlled clinical trials showing this do not exist in most cases. In considering the use of relatively large doses (within the recommended dosage range), sufficient benefit should be anticipated to offset the potential increased risk of GI toxicity (druginfonet, 2001).

1.6.2 Renal effects:

As with other NSAIDs, long-term administration of ibuprofen to animals has resulted in renal papillary necrosis and other abnormal renal pathology. In humans, there have been reports of acute interstitial nephritis with hematuria, proteinuria, and occasionally nephrotic syndrome (druginfonet, 2001).

A second form of renal toxicity has been seen in patients with prerenal conditions leading to a reduction in

renal blood flow or blood volume, where the renal prostaglandins have a supportive role in the maintenance of renal perfusion. In these patients administration of NSAID, may cause a dose dependent reduction in prostaglandin formation and may precipitate overt renal decompensation. Patients at greatest risk of this reaction are those with impaired renal function, heart failure, liver dysfunction, those taking diuretics and the elderly. Discontinuation of NSAID therapy is typically followed by recovery to the pretreatment state (druginfonet, 2001).

Those patients at high risk who chronically take Ibuprofen should have renal function monitored if they have signs or symptoms, which may be consistent with mild azotemia, such as malaise, fatigue, loss of appetite, etc. Occasional patients may develop some elevation of serum creatinine and blood urea nitrogen (BUN) levels without signs or symptoms (druginfonet, 2001).

Since primarily the kidneys eliminate ibuprofen, patients with significantly impaired renal function should be closely monitored and a reduction in dosage should be anticipated to avoid drug accumulation. Prospective studies

on the safety of ibuprofen in patients with chronic renal failure have not been conducted (druginfonet, 2001).

1.6.3 Liver Effects:

As with other NSAIDs, borderline elevations of one or more liver function tests may occur in up to 15% of patients. These abnormalities may progress, may remain essentially unchanged, or may be transient with continued therapy. The GPT (Glutamic Pyruvic Transaminase) test is probably the most sensitive indicator of liver dysfunction. Meaningful (3 times the upper limit of normal) elevations of GPT or GOT (Glutamic Oxaloacetic Transaminase) occurred in controlled clinical trials in less than 1% of patients. A patient with symptoms and/or signs suggesting liver dysfunction, or in whom an abnormal liver test has occurred, should be evaluated for evidence of the development of more severe hepatic reactions while on therapy with ibuprofen. Severe hepatic reactions, including jaundice and cases of fatal hepatitis, have been reported with ibuprofen as with other NSAIDs. Although such reactions are rare, if abnormal liver tests persist or worsen, if clinical signs and symptoms consistent with liver disease

develop, or if systemic manifestations occur (e.g., eosinophilia, rash, etc.), Ibuprofen should be discontinued (druginfonet, 2001).

1.6.4 Hemoglobin Levels:

In cross-study comparisons with doses ranging from 1200 mg to 3200 mg daily for several weeks, a slight dose-response decrease in hemoglobin/hematocrit was noted. This has been observed with other NSAIDs; the mechanism is unknown. However, even with daily doses of 3200 mg, the total decrease in hemoglobin usually does not exceed 1 gram; if there are no signs of bleeding, it is probably not clinically important (druginfonet, 2001).

In two postmarketing clinical studies the incidence of a decreased hemoglobin level was greater than previously reported. Decrease in hemoglobin of 1 gram or more was observed in 17.1% of 193 patients on 1600 mg Ibuprofen daily (osteoarthritis), and in 22.8% of 189 patients taking 2400 mg of Ibuprofen daily (rheumatoid arthritis). Positive stool occult blood tests and elevated serum creatinine levels were also observed in these studies (druginfonet, 2001).

1.6.5 Aseptic Meningitis:

Aseptic meningitis with fever and coma has been observed on rare occasions in patients on Ibuprofen therapy. Although it is probably more likely to occur in patients with systemic lupus erythematosus and related connective tissue diseases, it has been reported in patients who do not have an underlying chronic disease. If signs or symptoms of meningitis develop in a patient on Ibuprofen, the possibility of its being related to ibuprofen should be considered (druginfonet, 2001).

1.7 Drug Interactions:

1.7.1 Coumarin-type anticoagulants:

Several short-term controlled studies failed to show that Ibuprofen significantly affected prothrombin times or a variety of other clotting factors when administered to individuals on Coumarin-type anticoagulants. Ibuprofen like other NSAIDs can inhibit platelet aggregation but the effect is quantitatively less and of shorter duration than that seen with aspirin. Ibuprofen has been shown to prolong bleeding time (but within the normal range) in normal subjects. Because this prolonged bleeding effect may be exaggerated

in patients with underlying hemostatic defects, Ibuprofen should be used with caution in persons with intrinsic coagulation defects and those on anticoagulant therapy (druginfonet, 2001).

1.7.2 Aspirin:

Animal studies show that aspirin given with NSAIDs, including Ibuprofen yields a net decrease in anti-inflammatory activity with lowered blood levels of the non-aspirin drug. Single dose bioavailability studies in normal volunteers have failed to show an effect of aspirin on Ibuprofen blood levels. Correlative clinical studies have not been done (druginfonet, 2001).

1.7.3 Methotrexate:

Ibuprofen, as well as other NSAIDs, has been reported to competitively inhibit methotrexate accumulation in rabbit kidney slices. This may indicate that ibuprofen could enhance the toxicity of methotrexate. Caution should be used if Ibuprofen is administered concomitantly with methotrexate (druginfonet, 2001).

1.7.4 Furosemide:

Clinical studies, as well as random observations, have shown that Ibuprofen can reduce the natriuretic effect of furosemide and thiazides in some patients. This response has been attributed to inhibition of renal prostaglandin synthesis. During concomitant therapy with Ibuprofen, the patient should be observed closely for signs of renal failure, as well as to assure diuretic efficacy (druginfonet, 2001).

1.7.5 Lithium:

Ibuprofen produced an elevation of plasma lithium levels and a reduction in renal lithium clearance in a study of eleven normal volunteers. The mean minimum lithium concentration increased 15% and the renal clearance of lithium was decreased by 19% during this period of concomitant drug administration.

This effect has been attributed to inhibition of renal prostaglandin synthesis by Ibuprofen. Thus, when Ibuprofen and lithium are administered concurrently, subjects should be observed carefully for signs of lithium toxicity (druginfonet, 2001).

1.8 Pharmacokinetics:

Ibuprofen is rapidly absorbed when administered orally. Peak serum Ibuprofen levels are generally attained one to two hours after administration. With single doses up to 800 mg, a linear relationship exists between the amount of drug administered and the integrated area under the serum drug concentration vs. time curve. Above 800 mg, however, the area under the curve increases less than proportional to increases in dose. There is no evidence of drug accumulation or enzyme in action (druginfonet, 2001).

The administration of Ibuprofen tablets either under fasting conditions or immediately before meals yields quite similar serum Ibuprofen concentration-time profiles. When Ibuprofen is administered immediately after a meal, there is a reduction in the rate of absorption but no appreciable decrease in the extent of absorption. The bioavailability of Ibuprofen is minimally altered by the presence of food. A bioavailability study has shown that there was no interference with the absorption of ibuprofen when given in

conjunction with an antacid containing both aluminum hydroxide and magnesium hydroxide.

Ibuprofen is rapidly metabolized and eliminated in the urine. The excretion of Ibuprofen is virtually complete 24 hours after the last dose. The serum half-life is 1.8 to 2.0 hours (druginfonet, 2001).

1.8.1 Metabolism:

Studies have shown that following ingestion of the drug 45% to 79% of the dose was recovered in the urine within 24 hours as metabolite A (25%), (+)-2-4'-(2-hydroxy-2-methyl-propyl)-phenyl propionic acid and metabolite B (37%), (+)-2-4'- (2 carboxypropyl) phenyl propionic acid; the percentages of free and conjugated Ibuprofen were approximately 1% and 14%, respectively (druginfonet, 2001).

The metabolism of Ibuprofen enantiomers passes by the two phases, see figure (12) bellow (Mitchell, 1997).

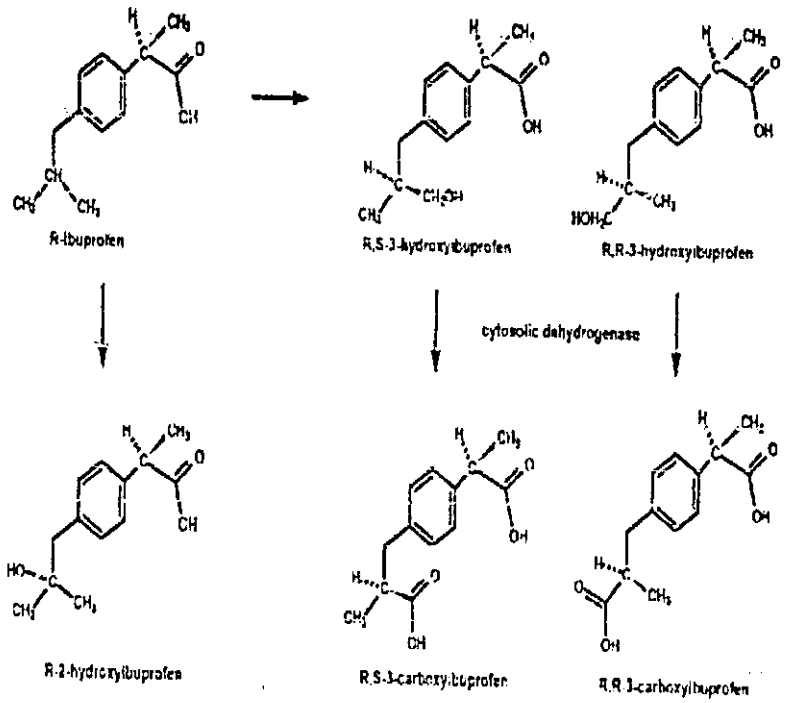


Figure 12: Oxidative metabolism of the enmantiomers of Ibuprofen in humans. When the substrate is R-Ibuprofen, cytochrome P450 produces R-2-OHIbu and two diasteriomeric 3-OHIbus designated R, S-and R, R-3-OHIbu. For S-ibuprofen, the corresponding products are S-2-OHIbu S, S-3-OHIbu and S, R-OHIbu. In vivo, the 3-OHIbus undergo nonmicrosomal dehydrogenation to the corresponding 3-carboxyibuprofens.

1.9 Drug life history; General approach:

Following oral administration of a drug product, the time course of the drug in the body can be broadly divided in three successive phases seen in figure (13) bellow:

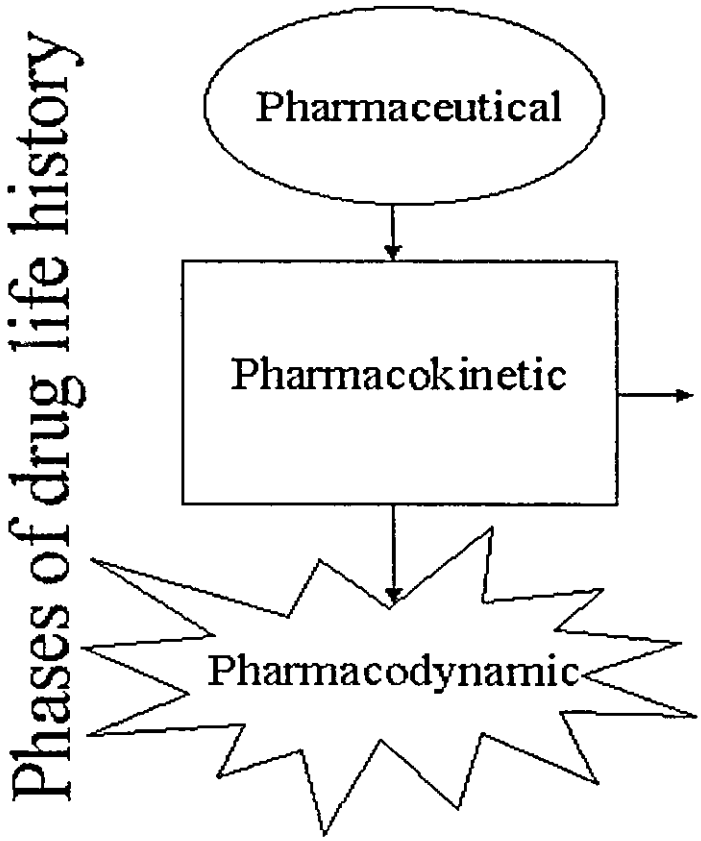


Figure 13: Schematic depiction of the main phases involved in the time course of a drug in the body.

The pharmaceutical phase includes all the processes that lead to drug release from the formulation, the pharmacokinetic phase includes absorption and disposition, and the pharmacodynamic phase includes all the pharmacological responces to the drug (Hadjiioannou, 1993).

The release of the drug from the conventional solid dosage form consists of a succession of two processes, (1) disintegration of the drug product, and (2) dissolution of

drug in the gastrointestinal fluids. In fact disintegration proceeds much faster than dissolution and, therefore, dissolution is the rate-determinating step in the absorption of the drug from solid drug product (Hadjiioannou, 1993).

The rate of dissolution of powder, dm/dt, is described by the Noyes/Whitney equation, which is based on Fick's first law of diffusion:

$dm/dt = kA (C_s - C)$----------equation (1).

Where *m* is the amount of drug dissolved at time *t* from the commencement of the experiment, *A* is the surface area of the drug particle, *Cs* is the solubility of the drug in the dissolution medium, *C* is the concentration of the drug at time *t*, and *k* is the intrinsic dissolution rate constant. The later parameter is a mass transfer coefficient defined by the underlying dissolution mechanism. For example in the diffusion layer model, dissolution is controlled by the diffusion of the drug in a stagnant liquid layer surrounding the drug particle, and ***K*** is given in units of cm/s by the equation:

K= D/h----------equation (2).

Where D is the diffusion coefficient of the drug (cm^2/s) and h is the thickness of the diffusion layer (cm). By integrating equation (1) it becomes in terms of concentration:

$C = Cs\ (1-e^{-K_d t})$----------equation (3).

Where $K_d = k\ (A/V)$, and V is the volume of dissolution medium.

1.9.1 Absorption:

The movement of drugs across the gastrointestinal (GI) membrane is known, as gastrointestinal absorption the driving force of the drug transport is the thermal movements of the drug molecules. Assuming a uniform concentration gradient across the GI membrane, the Fick's diffusion equation can be applied to describe the rate of drug penetration, dQ/dt:

$dQ/dt = P.A_m.(C_g-C_b)$----------equation(4).

Where dQ is the amount of drug diffusing per unit time (dt), P is the permeability coefficient of drug, A_m is the total membrane area and (C_g-C_b) is the concentration gradient between the GIU and the blood compartment. The

permeability coefficient is a constant defined by the relation:

P = [partition coefficient * Diffusion coefficient/ Thickness of the membrane].

The permeability coefficient is a measure of the drugs lipophilicity (Hadjiioannou, 1993).

1.9.2 Rate of dissolution versus absorption:

In case of drugs administered as suspensions or solids the rate of dissolution can be an important component of the overall rate of appearance of drug in the circulation figure (14).

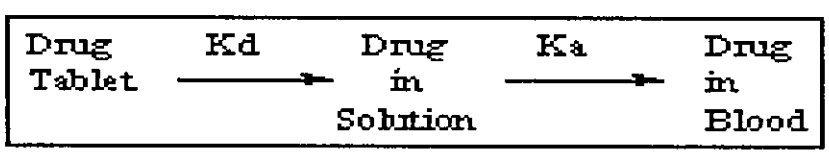


Figure 14: K_d = dissolution constant; K_a = absorption constant.

1.9.3 Binding:

Once a drug has entered the general circulation, either by intravenous administration or by absorption, it may gain access to various tissues and body fluids within the body. The distribution process depends on the physicochemical properties of the drug which determine (the ability of drug

molecules to pass through the body membranes and the binding of the drug to plasma proteins. The interaction of drugs with plasma proteins is a reversible process and its importance lies in the fact that the protein–bound drug doesn't cross the cellular protein. Thus only the unbound fraction of the drug can reach the drug receptors and the eliminating organs through the distribution process. However, one should keep in mind that the binding of drugs to proteins is a dynamic process and the bound and free drugs are permanently in equilibrium, since the binding reaction is rapidly reversible seen in the Figure (15) below (T. P. Hadjiioannou, 1993).

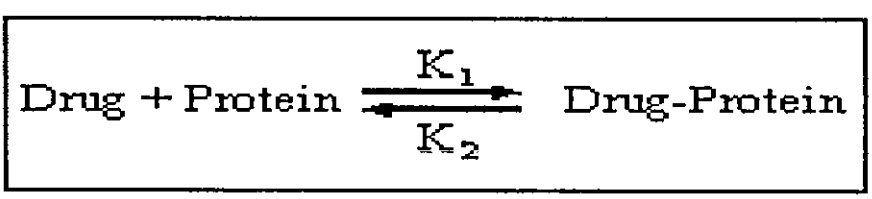


Figure 15: drug –protein-binding equilibrium.

Drug and protein associate and dissociate almost instantaneously so the Ka is constantly satisfied as drug or protein is added or removed. The association constant Ka = K_1 / K_2.

Protein is usually plasma albumin, but may also be alpha-1-acid glycoprotein, only free drug exerts diffusion pressure across most membrane barriers, therefore, the fraction of drug that is "unbound" (free) is important. It depends on:

- Drug concentration
- Protein concentration
- Association constant

$F_U = 1/[1+K_a*fu_p*P_t]$----------equation (5).

Where Fu = fraction unbound, K_a = association constant, fu_p = fraction of binding sites unoccupied and p_t = protein concentration. The formula holds at drug concentrations in therapeutic range for most drugs (Merck, 2003.

1.9.4.1 Tissue binding affinity:

Predilections for binding to:

Specific proteins in tissue, accumulation in endocytotic vesicles (e.g., gentamicin in renal proximal tubule), binding to nucleic acids, binding to calcium or accumulation in lipid

depots will do the following, and subsequently affect the drug's bioavailability to the site of action:

- ❖ Increase the concentration in the specific tissue relative to the plasma concentration
- ❖ "Pull" drug out of the plasma
- ❖ Serve as reservoirs of drug to lengthen total time the drug is in the body
- ❖ Make the calculated "volume of distribution" appear to be larger than it really is, in some cases exceeding the total body water.

When drug is both plasma protein bound and tissue bound, effects on distribution become very complex (Merck, 2003).

According to Figure (13) and since absorption, distribution and elimination are proceeding concurrently, the drug is in a dynamic state within the body, and its concentration changes continuously with time. Hence, pharmacokinetic models are described by equations that describe the drug level in the body as a function of time. Basically, the development of the appropriate equation relies on the characteristics of the model considered. For

example equation (4) adheres to the model depicted in figure (16).

(Rate of change of drug in systemic circulation) = Rate of absorption – Rate of elimination)----------equation (6).

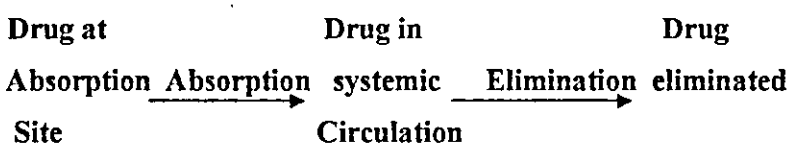


Figure 16: simple pharmacokinetic model.

However, the rewriting of equation (4) in the form of differential equation requires additional information about (1) the order of the rate of the processes and (2) the type of the distribution model for the drug considered. A one compartment model can be represented as in Figure (17):

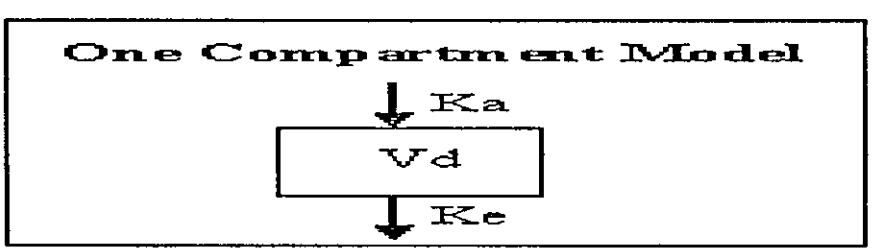


Figure 17: one compartment model

The one compartment open model treats the body as one homogeneous volume in which mixing is instantaneous. Input and output are from this one volume. In the majority

of cases when conventional dosage forms are examined, first order input is operating and equation (5) below is operating:

$dQ/dt = K_aQ_g - K_{el}Q$----------equation (7).

Where Q is the amount of the drug in the compartment and Q_g is the amount of drug in the GI. This equation can be integrated to give the well-known Bateman equation:

$C = FQ_0K_a * (e^{-K_{el}t} - e^{-K_at}) / [V * (K_a-K_{el})]$---equation (8).

Where *C* is the concentration of the drug in the body at time *t*, K_a and k_{el} are the first order absorption and elimination rate constants, respectively, *F* is the fraction of dose Q_0 absorbed, and V is the volume of distribution. Plot of C vs t gives the normal biexponential function.

The therapeutic window is the range of plasma drug concentrations with a high probability of therapeutic success equation (9).

Therapeutic Index = TD_{50} / ED_{50} _____equation (9).

Where TD_{50} is the minimum toxic dose that kills 50% of study population and ED_{50} is the minimum dose that shows therapeutic effects to 50% of study population. The

therapeutic range, peak time of action, and toxic level of Ibuprofen are 10-50 µg/mL, 1-2hr, ">"100 µg/mL respectively.

1.9.5 Bioavailability:

Extent to which, and sometimes the rate at which the active moiety (drug or metabolite) enters systemic circulation, thereby gaining access to the site of action.

The physicochemical properties of a drug govern its absorptive potential, but the properties of the dosage form (which partly depend on its design and manufacture) can largely determine drug bioavailability. Differences in bioavailability among formulations of a given drug can have clinical significance. Thus, the concept of equivalence among drug products is important in making clinical decisions (Aulton, 1999).

Chemical equivalence refers to drug products that contain the same compound in the same amount and that meet current official standards; however, inactive ingredients in drug products may differ. Bioequivalence refers to chemical equivalents that show insignificant difference (≤20%) in any of the following parameters: peak height concentration, time of peak height concentration and

areas under plasma concentration time curves (Aulton, 1999).

Therapeutic equivalence refers to drug products that, when administered to the same person in the same dosage regimen, provide essentially the same therapeutic effect or toxicity. Bioequivalent products are expected to be therapeutically equivalent. Therapeutic problems (eg, toxicity, lack of efficacy) are encountered most frequently during long-term therapy when a patient who is stabilized on one formulation is given a nonequivalent substitute.

1.9.5.1 past bioavailability problems:

There are a number of examples of drugs products, which have exhibited bioavailability problems in the past. These examples are all pre-1976. More attention is now being given to formulation development during drug development (Boomer, 2003).

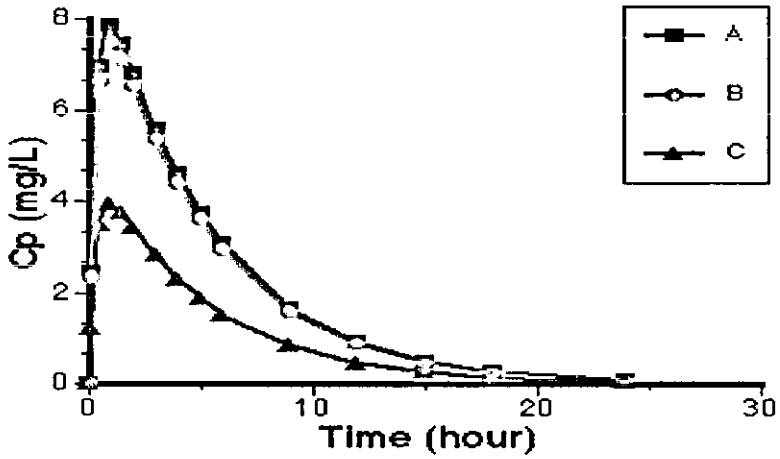


Figure 18: Plot of Cp versus Time

Chlorpropamide. With three products tested the peak plasma concentration after one brand was less than 1/2 the peak after the other two products see Figure (18).

Digoxin. The text reports a number of bioavailability problems with digoxin. One example is particularly interesting. Doctors in Israel noticed 15 cases of digoxin toxicity between Oct/Dec 1975 with almost no reports for the same period the previous year. It was found that the local manufacturer had changed the formulation to improve dissolution without telling the physicians. Urinary data suggested a two-fold increase in availability of the new formulation.

Phenytoin. Again there are a number of examples in the text. One report described an incidence of phenytoin intoxication in Australia in 1968 and 1969. Apparently the tablet diluent was changed from calcium sulfate to lactose. Later studies showed that the bioavailability was higher in the dosage form containing lactose.

1.9.5.2 Reasons for bioequivalence requirements:

The FDA may decide to require bioavailability studies for a variety of reasons including:

- Results from clinical studies indicate that different drug products produce different therapeutics results.
- Results from bioavailability studies indicate that different products are not bioequivalent.
- Drug has a narrow therapeutic range.
- Low solubility and/or large dose.
- Absorption is considerably less than 100%.
- General public complain from inefficacy of some formulations (Boomer, 2003).

Sometimes therapeutic equivalence may be achieved despite differences in bioavailability. For example, the therapeutic index (ratio of the maximum tolerated dose to the minimum effective dose) of penicillin is so wide that moderate blood concentration differences due to bioavailability differences in penicillin products may not affect therapeutic efficacy or safety.

In contrast, bioavailability differences are important for a drug with a relatively narrow therapeutic index. The physiologic characteristics of the patient affect bioavailability. Absorption rate is important because even when a drug is absorbed completely, it may be absorbed too slowly to produce a therapeutic blood level quickly enough or so rapidly that toxicity results from high drug concentrations after each dose. The estimation of bioavailability (F) can be achieved by integrating equation (6) where:

$$AUC_{0 \longrightarrow \infty} = \int Cdt = \int FQ_0K_a * (e^{-K_{el}t} - e^{-K_at})/[V*(K_a-K_{el})] = [FQ_0/V.K_{el}] \quad \text{equation (10).}$$

So it is obvious that there is a direct proportionality between AUC & F (Hadjiioannou, 1993). But since it is not

easy to withdraw about 12 blood samples from each subject of the 72 that were included in the study we only withdraw three samples where the third one is at a time $\leq T_{max}$ for Ibuprofen and subsequent comparisons will be based upon plasma concentrations rather than area under concentration time curves.

1.9.5.3 Bioavailability study characteristics:

The evaluation of a drug product bioavailability study involves the consideration of various factors.

1.9.5.3.1 Drug product:

The drug substance in each product must be the same. Bioavailability studies are conducted to compare two or more products containing the same chemical substance. We can't compare different chemical substances. The apparent volume of distribution and kel can be quite different for different drug substances, thus no interpretation of the results is possible. The first rule of bioavailability testing is that you compare the drug products with the same drug in each dosage form.

The only time that this rule is relaxed is in the case of pro-drug administration. A pro-drug is a compound, which

will form the drug of interest in the body. In this case it may be appropriate to compare the delivery of a dosage form containing the drug with another dosage form containing a pro-drug. This testing is generally conducted to evaluate the usefulness of the pro-drug, rather than a strict comparison of the drug products. Once the usefulness of the pro-drug is demonstrated comparisons between dosage forms all containing the pro-drug should be undertaken to evaluate the drug product performance.

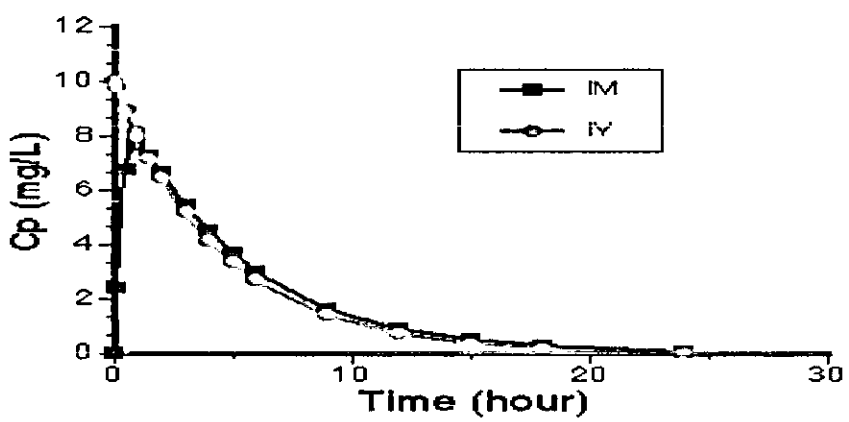


Figure 19: Plot of Cp versus Time after intrvenous and intramascular administration

NOTE: AUC are the same.

Usually the comparison is made between two (or more) similar products, containing exactly the same chemical substance. However, different dosage forms can be compared (when they contain the same drug). For

be compared (when they contain the same drug). For example we could compare an IM dosage form with an IV one, figure (19).

By calculating the AUC values we can determine the absolute bioavailability of the IM dosage form. In this case it appears to be close to 100%. The rate of absorption for the IM dose can be determined also, but of course no comparison is possible.

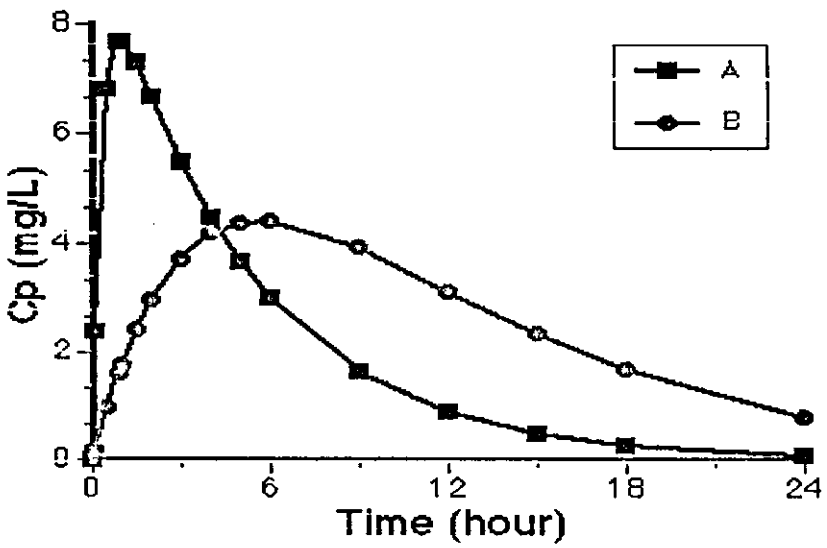


Figure 20: Plot of Cp versus Time for A and B With B having Slower Absorption.

Alternately we could compare brand A tablet with brand B tablet or capsule.

By comparing the AUC values and ka values we can make comparisons concerning both the extent and rate of

absorption. In this case A appears to be faster than B but the extent of absorption doesn't appear to be different figure (20).

1.9.5.3.2 Subjects

A number of factors are of concern; health, age, weight, enzyme status, and number of subjects.

1. Health:

Usually a study is designed so that each subject takes each product in turn. Thus the effect of the individual subject can be eliminated or reduced. Such a study design is called a cross-over design. Even though each subject will act as their own control it is usually best to have subjects of similar kinetic characteristic so that major variations are not introduced. Thus healthy volunteers are often preferred by drug product evaluation studies. Informed consent should be obtained from each volunteer and some biochemical and medical examination will be used to confirm their medical state. For some drugs there may be special disease states, which may cause the exclusion of some volunteers. For example, in one study examining propranolol products,

healthy volunteers with a past history of asthma were excluded from this study (Boomer, 2003).

2. Age:

Age can have a significant effect on drug harmacokinetics. Elderly patients and young children can have quite different kinetics compared with young adults. In the interest of a better-matched group, subjects between the ages of 18 to 35 years are preferred. Kinetic changes usually aren't important until age greater than 60.

3. Weight:

The apparent volume of distribution is usually proportional to weight in subjects of normal weight for height. However, in overweight (or underweight) subjects the V in L/kg maybe somewhat different. Again to better match the subjects, normal weights are preferred.

4. Enzyme status:

Smokers or subjects taking certain other drugs may have altered kinetics for the drug of interest. This can be caused by alteration of enzyme activity or by drug-drug interactions. These effects add complications to a study and an attempt is usually made to minimize these factors.

1.9.5.3.3 Design:

Usually a complete cross-over design is used. With this design each subject receives all products with a wash-out period between each dose administration; the table bellow shows the design for three drug products comparative study.

	Week 1	Week 2	Week 3	
Group 1	A	B	C	
Group 2	B	C	A	
Group 3	C	A	B	
Group 4	A	C	B	
Group 5	C	B	A	
Group 6	B	A	C	For three Products

Table (4): Three-Product Example.

When more than 3 or 4 products are involved it has been suggested that a different design is used (Boomer, 2003).

1.9.5.3.4 Number:

The number of subjects included in the study should be sufficient to see any real (may be 20% variation) differences

in bioavailability. Usually 10 - 20 subjects are used in these studies. In clinical studies where the end-point is some clinical response, much larger numbers are required because of the greater variability in clinical response (Medscape; Portolés; Vargas, 2002).

CHAPTER TWO

METHODOLOGY AND RESULTS

2. SUBJECTS AND METHODOLOGY:

2.1 Subjects:

Using published data on Ibuprofen as a basis, sample estimation was made. A sample of 24 subjects for each drug formulation is a reliable sample size (Medscape, 2002). Three blood samples were withdrawn from each subject (total of 24 subjects); 12 males and 12 females with no relevant clinical abnormalities were included in the trial and the averages of their age, weight, height and body mass index were 20.667, 65.292, 1.6929 and 22.750 respectively. All participants provided signed informed consent.

2.2 Methods:

Subjects were admitted to the central medical laboratory on three occasions for the administration of a single oral dose of each of the study drugs. The study drugs are: 1-Ibuprofen (locally made), 2-Advil® made in USA) and 3-Nurofen® (made in UK). In order to determine Ibuprofen blood concentration, and since it is

harder to recruit subjects for longer times, 3 blood samples were drawn (approximately 5 ml each) via a syringe during each period at the following times: 0.5 hr, 1 hr and 1.5 hr after drug administration. After centrifugation (within 20 minutes following blood collection) for 10 minutes at (3000 rpm), the plasma was separated and the labeled samples were frozen at -40°C in a vertical position and stored at this temperature until they were analyzed. The following conditions were established: the subjects were admitted to the Unit at the morning before drug administration, fasting was 1 hr before administration of the formulation.

2.3 Drugs and Formulations:

2.3.1 Test formulation:

- Nurofen® 400 mg Tablets formulated by Boots Healthcare Int.Nottingham, England and Imported by Abic Marketing Ltd.Lot 9kk,DOM 10/2001 and Exp.date is 10/2004.

- Local brand: 400 mg Tablets formulated by a local Pharmaceutical Company, Batch No.030155 and Exp. Date 1/2008)
- Advil®: 400 mg tablets made by Whitehall-Robins healthcare, Madison, NJ 07940, US patent No.5, 087,454.Made in USA, Exp.date 09/2004,Batch No.3020129.

The medication was taken with 150 ml of water. Administration of the formulation was by the researcher at the specified time.

2.4 Apparatus and Equipments:

1-HPLC/UV system (Model SPD-M10AVP)
NO.C20903502076J7, SCL-10A_{VP} SHIMADZU (SYSTEM CONTROLLER) &LC-10 AT_{VP} SHIMATZU (LIQUID CHROMATOGRAM)

2- Volumetric-flasks, 10, 50, 100 and 1000 ml.

3- Filter membranes 0.45 µm, 0.2 µm.

4- micro-pipettes.

2.4.1 HPLC conditions:

1- Column: reversed-phase

Spherisorb C18. SelectB 5µ (125*4.6) mm.

2- Detecter: U.V spectrophotometer at 220 nm.

3- Flow rate: 1 ml/min.

4- Mobile phase: 100% Mobile Phase solution.

2.5 Chemicals and reagents:

1- Phosphoric acid.

2- Acetonitrile HPLC.

3- Water for HPLC.

2.5.1 Standards preparation:

1000 ppm standard solution of Ibuprofen (USP St.d) was prepared by dissolution of 100 mg in 40 ml of HPLC acetonitrile and diluted to 100 ml by 0.01 M phosphoric acid. The other 9 standards (500 ppm, 100 ppm, 75 ppm, 50 ppm, 25 ppm, 10 ppm, 5 ppm, 1 ppm and 0.5 ppm) were prepared by serial dilution.

2.5.2 Mobile phase and Buffer preparation:

1. Buffer solution.

0.7 ml of phosphoric acid was diluted to 1000 ml with HPLC water.

2. Mobile phase:

400ml of HPLC acetonitrile was diluted to 1000 ml with 0.01 M of phosphoric acid (BP 98).The PH the mobile phase was measured to be 3.3.

2.6 Sample Preparation:

Plasma samples were allowed to thaw at room temperature and 0.5 ml aliquots were processed together with 2 ml of HPLC acetonitrile and diluted to 5 ml with 0.01 M phosphoric acid, centrifuged at 3000 rpm for 10 minutes and separated.

2.7 Procedure:

20 µL of each of standard solutions was injected into the HPLC and the retention time was monitored, it was 13 minutes, then 20 µL of each sample of the three for each subject for each formulation was injected also and the peak appearance was monitored after 13 minutes of injection.

2.8 Active ingredient and dissolution test assay:

2.8.1 Active ingredient assay:

Six tablets of each local medication (Batch No.030155 and Exp. Date 1/2008) and Nurofen (Lot: 9kk and Exp Date: 10/2004) formulations were weighed and grinded; the average weights were 0.6185 and 1.045 gm respectively.

154.625 and 261.25 mg of the local brand and Nurofen® were taken respectively and 1000 ppm and 500 ppm solutions of each were prepared and samples of 20 µL of each solution were injected and compared with the correspondent standard solution.

2.8.2 Dissolution test assay:

In vitro dissolution testing can serve one of several functions:

1. Act as a guide for formulation design and development.
2. Provide a measure of manufacturing process consistency.
3. Establish a relationship with in vivo performance.
4. Provide a regulatory approval criterion (J.B. Dressman, 1998).

Apparatus: USP Dissolution tester apparatus 6 (paddle) at 50 rpm.

Medium: 900 ml of phosphate buffer pH at 7.2 at $37C^{0}$.

Procedure: one tablet of local brand 400 mg was placed into each chamber to get a solution of 0.444 mg/ml concentration and the apparatus was operated for 60 minutes (U.S.P 24/NF19)

2.8.3 Assay for Ibuprofen:

1. Sample preparation:

At the end of dissolution period about 20 ml from each vessel was taken and 20 µL aliquot was filtered through 0.45 micrometer and injected, and the absorbance at 220 nm was measured.

2. Standard preparation: quantity of Ibuprofen RS standard (99.0% pure) equivalent to 40 mg was dissolved in 40 ml acetonitrile and diluted to 100 ml with 0.01 M of phosphoric acid to get 400 ppm Ibuprofen solution, then sample of 20 µL was filtered through 0.45 micrometer membrane and injected and the absorbance was found to be 0.579 Abs.U at 220 nm.

2.9 Data Display

Sub. No	P1*	C1*	P2*	C2*	P3*	C3*	P1	C1	P2
1	4.530	6.07762	6.170	8.19922	11.243	14.7620	0.939	1.43208	1.445
2	4.244	5.70763	6.530	8.66494	10.047	13.2147	1.003	1.51488	1.498
3	4.812	6.44243	7.292	9.65071	11.045	14.5058	0.708	1.13325	1.180
4	3.066	4.18370	4.946	6.61578	7.852	10.3752	0.639	1.04398	1.083
5	3.349	4.54981	4.999	6.68435	7.462	9.8706	0.705	1.12937	1.120
6	3.297	4.48254	4.779	6.39974	6.927	9.1785	0.833	1.29495	1.282
7	2.870	3.93014	4.704	6.30272	7.713	10.1953	0.659	1.06986	1.118
8	3.158	4.30272	4.577	6.13842	6.732	8.9263	0.415	0.75420	0.815
9	2.975	4.06598	4.375	5.87710	6.878	9.1151	0.411	0.74903	0.893
10	2.746	3.76973	4.578	6.13972	7.630	10.0880	0.788	1.23674	1.313
11	3.776	5.10220	5.553	7.40103	8.289	10.9405	0.692	1.11255	1.066
12	4.151	5.58732	6.386	8.47865	9.826	12.9288	0.839	1.30272	1.292
13	2.998	4.09573	4.614	6.18629	7.325	9.6934	0.678	1.09444	1.130
14	4.339	5.83053	6.476	8.59508	9.667	12.7232	0.835	1.29754	1.193
15	3.801	5.13454	6.034	8.02329	9.733	12.8085	0.462	0.81501	1.115
16	2.598	3.57827	3.998	5.38939	7.235	9.5770	0.674	1.08926	1.038
17	3.767	5.09056	5.979	7.95213	8.925	11.7633	0.662	1.07374	1.019
18	3.959	5.33894	6.186	8.21992	9.978	13.1255	0.704	1.12807	1.083
19	3.323	4.51617	5.538	7.38163	9.231	12.1591	0.642	1.04787	1.071
20	2.993	4.08926	5.023	6.71539	7.139	9.4528	0.780	1.22639	1.165
21	3.012	4.11384	5.012	6.70116	7.853	10.3765	0.596	0.98836	1.065
22	4.039	5.44243	6.214	8.25614	9.560	12.5847	0.665	1.07762	0.992
23	3.162	4.30789	4.790	6.41397	7.259	9.6080	0.970	1.47219	1.293
24	2.593	3.57180	4.321	5.80724	7.203	9.5356	0.784	1.23157	1.046

Sub. No	C2	P3	C3	P1**	C1**	P2**	C2**	P3**	C3**
1	2.08668	2.224	3.09444	4.230	5.68952	6.507	8.63519	10.012	13.1695
2	2.15524	2.236	3.10996	3.779	5.10608	5.815	7.73997	8.947	11.7917
3	1.74386	1.967	2.76197	4.155	5.59250	6.393	8.48771	9.836	12.9418
4	1.61837	1.748	2.47865	2.957	4.04269	4.548	6.10091	6.992	9.2626
5	1.66624	1.713	2.43338	3.064	4.18111	4.788	6.41138	7.367	9.7477
6	1.87581	2.332	3.23415	2.821	3.86675	4.478	6.01035	6.998	9.2704
7	1.66365	1.962	2.75550	2.775	3.80724	4.476	6.00776	6.869	9.1035
8	1.27167	1.665	2.37128	2.571	3.54334	3.896	5.25744	5.995	7.9728
9	1.37257	2.234	3.10737	2.826	3.87322	4.486	6.02070	6.902	9.1462
10	1.91591	2.265	3.14748	2.870	3.93014	4.416	5.93014	6.795	9.0078
11	1.59638	1.640	2.33894	3.118	4.25097	4.872	6.52005	7.382	9.7671
12	1.88875	2.139	2.98448	3.942	5.31695	6.259	8.31436	9.628	12.6727
13	1.67917	1.983	2.78266	3.351	4.55239	5.155	6.88616	7.932	10.4787
14	1.76067	1.865	2.63001	3.877	5.23286	6.154	8.17853	9.325	12.2807
15	1.65977	2.889	3.95472	3.549	4.80854	5.460	7.28072	8.667	11.4295
16	1.56016	1.597	2.28331	3.318	4.50970	5.351	7.13972	7.988	10.5511
17	1.53558	2.038	2.85382	4.085	5.50194	6.190	8.22510	9.379	12.3506
18	1.61837	2.709	3.72186	4.216	5.67141	6.487	8.60931	10.297	13.5382
19	1.60285	1.786	2.52781	3.773	5.09832	5.805	7.72704	8.932	11.7723
20	1.72445	1.793	2.53687	2.368	3.28072	3.759	5.08021	5.968	7.9379
21	1.59508	1.746	2.47607	3.180	4.33118	4.611	6.18241	6.987	9.2561
22	1.50065	1.576	2.25614	3.128	4.26391	4.965	6.64036	7.882	10.4140
23	1.89004	1.725	2.44890	3.643	4.93014	5.604	7.46701	8.623	11.3726
24	1.57050	1.395	2.02199	2.809	3.85123	4.466	5.99483	6.979	9.2458

Table 5: Peak heights (mAb. U) and concentrations (ppm) of different drugs in plasma samples at the three periods (0.5hr, 1hr and 1.5hr).

Note:

(P): peak height, (c): concentration of drug in the plasma, (1): after 1/2hr, (2): after 1hr, (3): after 1.5hr, (*): Nurofen®, (without asterisk): local brand, (**): Advil®

St,d.No.	Peak1 Hight.(mAu	St,d1	Conc.(ppm)	Peak2 Hight.(mAu)	St,d2	Con.(ppm)
1	77.681		100.0	678.623		1000.0
2	57.678		75.0	373.124		500.0
3	37.930		50.0	77.681		100.0
4	18.425		25.0	57.678		75.0
5	7.225		10.0	37.930		50.0
6	4.324		5.0	18.425		25.0
7	0.958		1.0	7.225		10.0
8	0.434		0.5	4.324		5.0
9				0.958		1.0
10				0.434		0.5

Table 6:Standards concentrations (ppm) and their correspondent peaks heights (mAbs. U).

Sub.No.	C1M*	C1F*	C2M*	C2F*	C3M*	C3F*	C1M	C1F
1	6.07762	4.09573	8.19922	6.18629	14.7620	9.6934	1.43208	1.09444
2	5.70763	5.83053	8.66494	8.59508	13.2147	12.7232	1.51488	1.29754
3	6.44243	5.13454	9.65071	8.02329	14.5058	12.8085	1.13325	0.81501
4	4.18370	3.57827	6.61578	5.38939	10.3752	9.5770	1.04398	1.08926
5	4.54981	5.09056	6.68435	7.95213	9.8706	11.7633	1.12937	1.07374
6	4.48254	5.33894	6.39974	8.21992	9.1785	13.1255	1.29495	1.12807
7	3.93014	4.51617	6.30272	7.38163	10.1953	12.1591	1.06986	1.04787
8	4.30272	4.08926	6.13842	6.71539	8.9263	9.4528	0.75420	1.22639
9	4.06598	4.11384	5.87710	6.70116	9.1151	10.3765	0.74903	0.98836
10	3.76973	5.44243	6.13972	8.25614	10.0880	12.5847	1.23674	1.07762
11	5.10220	4.30789	7.40103	6.41397	10.9405	9.6080	1.11255	1.47219
12	5.58732	3.57180	8.47865	5.80724	12.9288	9.5356	1.30272	1.23157

Sub.No.	C2M	C2F	C3M	C3F	C1M**	C1F**	C2M**	C2F**
1	2.08668	1.67917	3.09444	2.78266	5.68952	4.55239	8.63519	6.88616
2	2.15524	1.76067	3.10996	2.63001	5.10608	5.23286	7.73997	8.17853
3	1.74386	1.65977	2.76197	3.95472	5.59250	4.80854	8.48771	7.28072
4	1.61837	1.56016	2.47865	2.28331	4.04269	4.50970	6.10091	7.13972
5	1.66624	1.53558	2.43338	2.85382	4.18111	5.50194	6.41138	8.22510
6	1.87581	1.61837	3.23415	3.72186	3.86675	5.67141	6.01035	8.60931
7	1.66365	1.60285	2.75550	2.52781	3.80724	5.09832	6.00776	7.72704
8	1.27167	1.72445	2.37128	2.53687	3.54334	3.28072	5.25744	5.08021
9	1.37257	1.59508	3.10737	2.47607	3.87322	4.33118	6.02070	6.18241
10	1.91591	1.50065	3.14748	2.25614	3.93014	4.26391	5.93014	6.64036
11	1.59638	1.89004	2.33894	2.44890	4.25097	4.93014	6.52005	7.46701
12	1.88875	1.57050	2.98448	2.02199	5.31695	3.85123	8.31436	5.99483

Sub.No.	C3M**	C3F**	RESI1	FITS1	COEF1
1	13.1695	10.4787	0.551462	77.1295	-0.167801
2	11.7917	12.2807	-0.127203	57.8052	0.772973

3	12.9418	11.4295	- 0.550868	38.4809
4	9.2626	10.5511	-0.731534	19.1565
5	9.7477	12.3506	-0.336933	7.5619
6	9.2704	13.5382	0.626934	3.6971
7	9.1035	11.7723	0.352828	0.6052
8	7.9728	7.9379	0.215314	0.2187
9	9.1462	9.2561		
10	9.0078	10.4140		
11	9.7671	11.3726		
12	12.6727	9.2458		

Table 7: Concentrations (ppm) of the three drugs in plasma Samples at the three periods for both genders separately. Note: M for male and F for female, RESI1 stands for residuals and COEF1for coefficients.

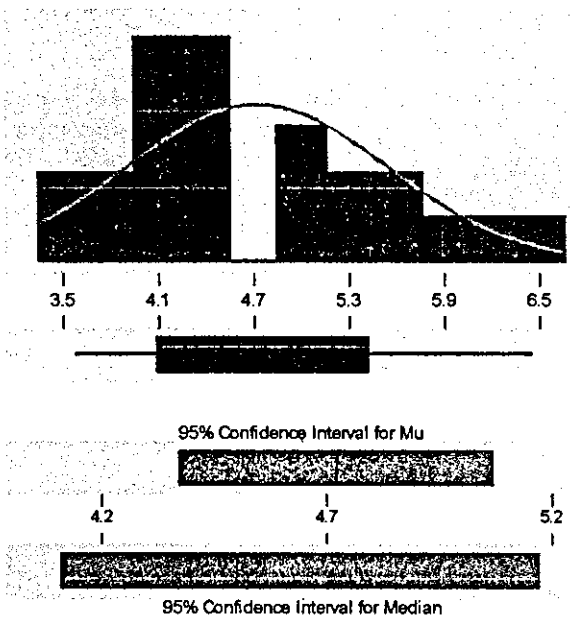
2.9.1 Descriptive Statistics:

Variable	N	Mean	Median	Tr. Mean	St. Dev	SE. Mean
C1*	24	4.721	4.499	4.695	0.824	0.168
C2*	24	7.175	6.708	7.143	1.134	0.232
C3*	24	11.146	10.376	11.083	1.802	0.368
C1	24	1.1382	1.1203	1.1387	0.1982	0.0405
C2	24	1.6897	1.6617	1.6875	0.2035	0.0415
C3	24	2.7630	2.6928	2.7425	0.4653	0.0950
C1**	24	4.551	4.420	4.557	0.723	0.148
C2**	24	6.952	6.763	6.961	1.092	0.223
C3**	24	10.603	10.446	10.591	1.675	0.342

Variable	Min	Max	Q1	Q3
C1*	3.572	6.442	4.091	5.417
C2*	5.389	9.651	6.215	8.215
C3*	8.926	14.762	9.585	12.787
C1	0.7490	1.5149	1.0534	1.2804
C2	1.2717	2.1552	1.5766	1.8470
C3	2.0220	3.9547	2.4373	3.1041
C1**	3.281	5.690	3.887	5.201
C2**	5.080	8.635	6.013	8.069
C3**	7.938	13.538	9.248	12.158

Table 8:Descriptive statistics for concentration Columns in table 5 Note: Tr.mean stands for trimmed mean, St. Dev stands for standard deviation, and SE.mean stands for standard error mean.

Descriptive Statistics

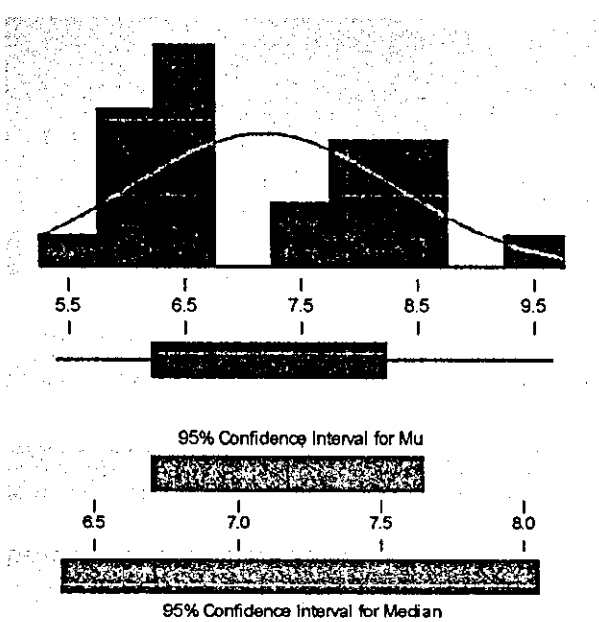


Variable: C1*

Anderson-Darling Normality Test	
A-Squared:	0.579
P-Value:	0.118
Mean	4.72132
StDev	0.82412
Variance	0.679174
Skewness	0.420926
Kurtosis	-1.09284
N	24
Minimum	3.57180
1st Quartile	4.09088
Median	4.49935
3rd Quartile	5.41656
Maximum	6.44243
95% Confidence Interval for Mu	
4.37333	5.06932
95% Confidence Interval for Sigma	
0.64052	1.15604
95% Confidence Interval for Median	
4.11070	5.16995

Figure 21:descriptive statistics for concentrations of Nurofen® in plasma samples for the 24 subjects after 0.5hr

Descriptive Statistics

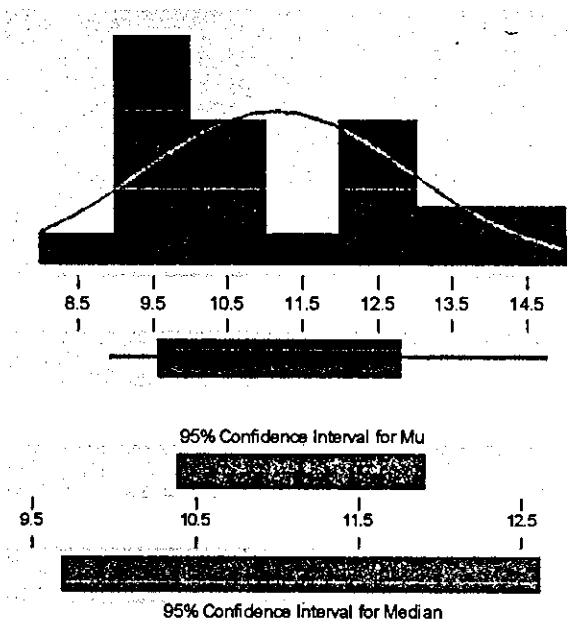


Variable: C2*

Anderson-Darling Normality Test	
A-Squared:	0.692
P-Value:	0.062
Mean	7.17475
StDev	1.13425
Variance	1.28653
Skewness	0.355977
Kurtosis	-1.10449
N	24
Minimum	5.38939
1st Quartile	6.21540
Median	6.70828
3rd Quartile	8.21475
Maximum	9.65071
95% Confidence Interval for Mu	
6.69580	7.65370
95% Confidence Interval for Sigma	
0.88156	1.59108
95% Confidence Interval for Median	
6.38293	8.05377

Figure 22:descriptive statistics for concentrations of Nurofen® in plasma samples for the 24 subjects after 1hr

Descriptive Statistics

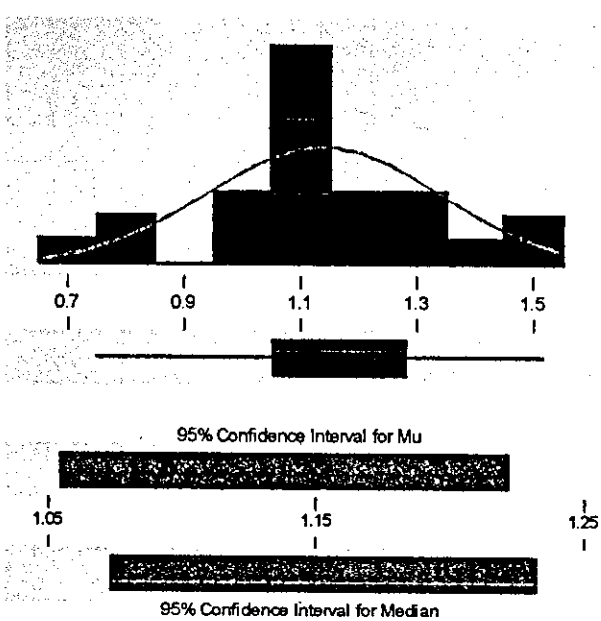


Variable: C3*

Anderson-Darling Normality Test	
A-Squared:	0.983
P-Value:	0.011
Mean	11.1462
StDev	1.8018
Variance	3.24641
Skewness	0.488998
Kurtosis	-1.20444
N	24
Minimum	8.9263
1st Quartile	9.5847
Median	10.3759
3rd Quartile	12.7872
Maximum	14.7620
95% Confidence Interval for Mu	
10.3854	11.9070
95% Confidence Interval for Sigma	
1.4004	2.5275
95% Confidence Interval for Median	
9.6786	12.6087

Figure 23:descriptive statistics for concentrations of Nurofen® in plasma samples for the 24 subjects after 1.5hr

Descriptive Statistics



Variable: C1

Anderson-Darling Normality Test	
A-Squared:	0.501
P-Value:	0.187
Mean	1.13815
StDev	0.19820
Variance	3.93E-02
Skewness	-1.3E-01
Kurtosis	-3.4E-01
N	24
Minimum	0.74903
1st Quartile	1.05337
Median	1.12031
3rd Quartile	1.28040
Maximum	1.51488
95% Confidence Interval for Mu	
1.05446	1.22185
95% Confidence Interval for Sigma	
0.15405	0.27803
95% Confidence Interval for Median	
1.07307	1.23247

Figure 24:descriptive statistics for concentrations of the local brand in plasma samples for the 24 subjects after 0.5hr

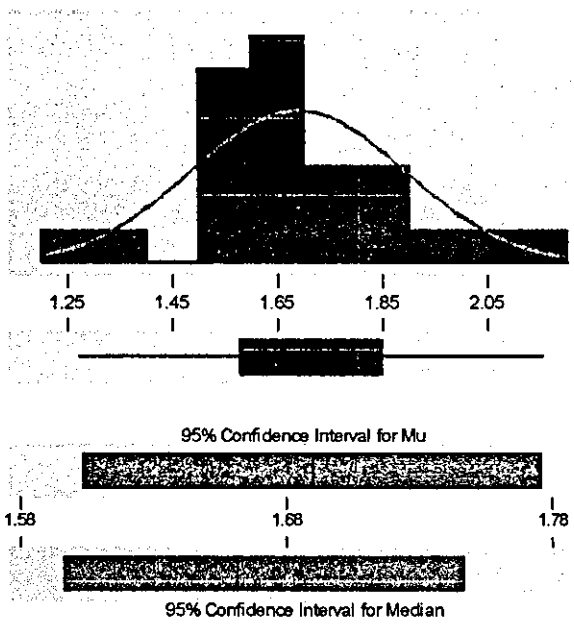


Figure 25:descriptive statistics for concentrations of the local brand in plasma samples for the 24 subjects after 1hr.

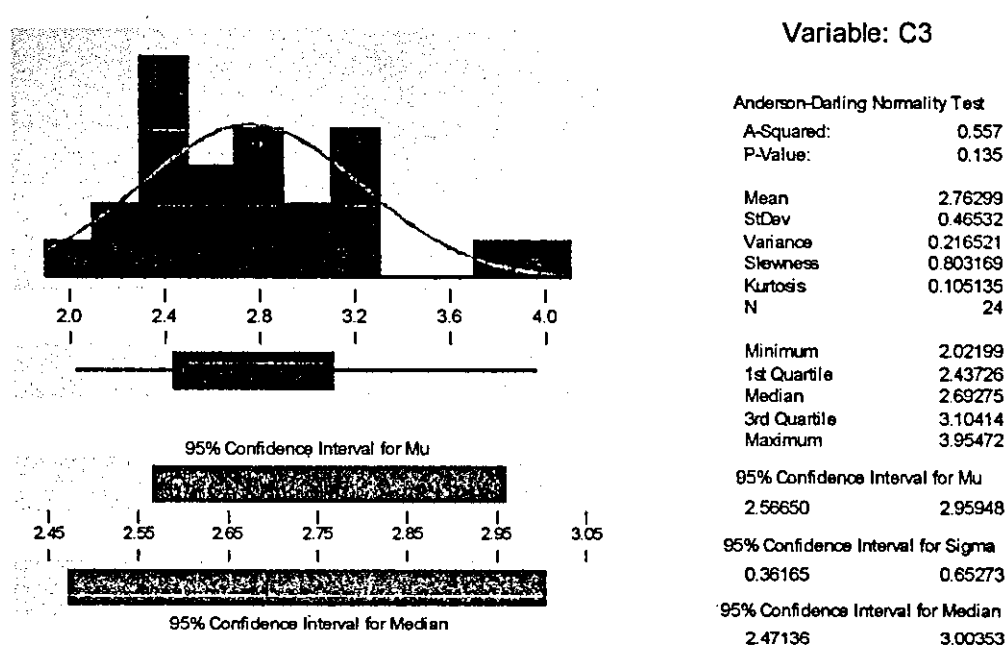
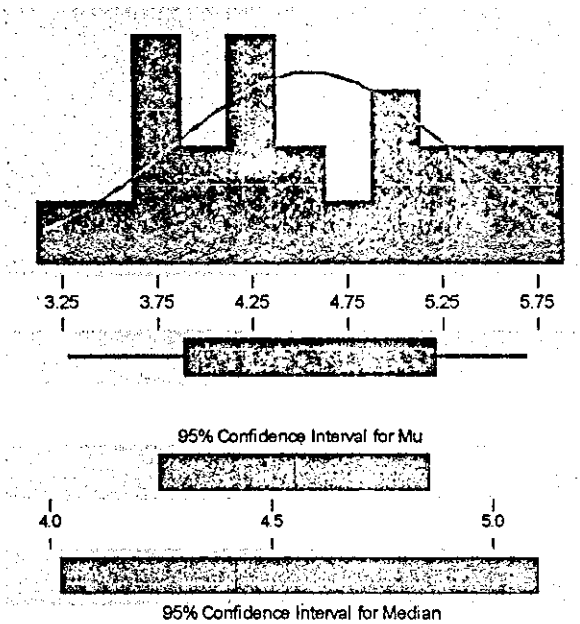


Figure 26:descriptive statistics for concentrations of the local brand in plasma samples for the 24 subjects after 1.5hr

Descriptive Statistics

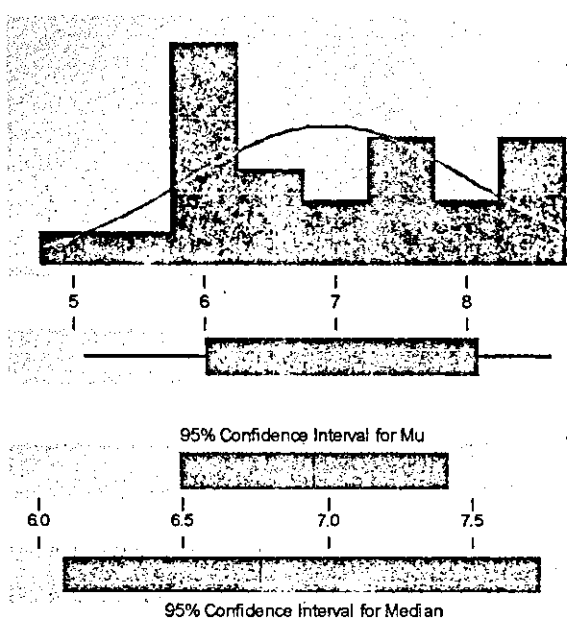


Variable: C1**

Anderson-Darling Normality Test	
A-Squared:	0.462
P-Value:	0.236
Mean	4.55137
StDev	0.72269
Variance	0.522281
Skewness	0.120351
Kurtosis	-1.33794
N	24
Minimum	3.28072
1st Quartile	3.88745
Median	4.42044
3rd Quartile	5.20116
Maximum	5.68952
95% Confidence Interval for Mu	
4.24620	4.85653
95% Confidence Interval for Sigma	
0.56168	1.01376
95% Confidence Interval for Median	
4.02319	5.09966

Figure 27:descriptive statistics for concentrations of Advil® in plasma samples for the 24 subjects after 0.5hr

Descriptive Statistics

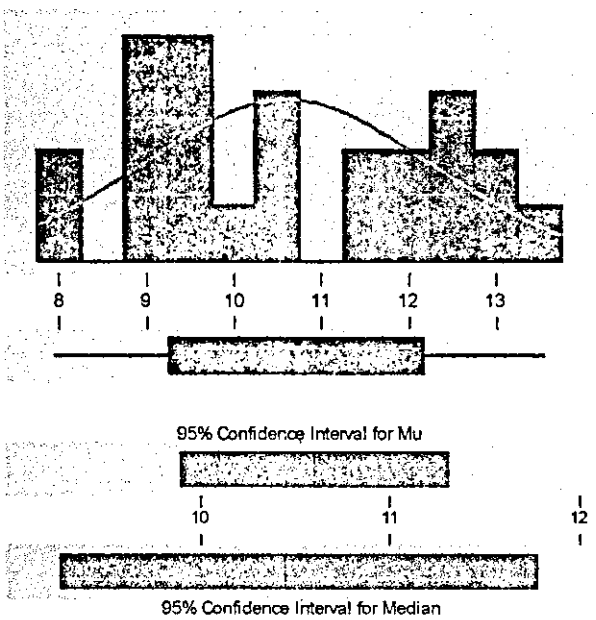


Variable: C2**

Anderson-Darling Normality Test	
A-Squared:	0.579
P-Value:	0.117
Mean	6.95197
StDev	1.09202
Variance	1.19250
Skewness	0.106277
Kurtosis	-1.35664
N	24
Minimum	5.08021
1st Quartile	6.01294
Median	6.76326
3rd Quartile	8.06889
Maximum	8.63519
95% Confidence Interval for Mu	
6.49085	7.41309
95% Confidence Interval for Sigma	
0.84873	1.53184
95% Confidence Interval for Median	
6.08701	7.72928

Figure 28:descriptive statistics for concentrations of Advil® in plasma samples for the 24 subjects after 1hr

Descriptive Statistics



Variable: C3**

Anderson-Darling Normality Test	
A-Squared:	0.582
P-Value:	0.118
Mean	10.6034
StDev	1.6750
Variance	2.80562
Skewness	0.173348
Kurtosis	-1.32614
N	24
Minimum	7.9379
1st Quartile	9.2484
Median	10.4463
3rd Quartile	12.1585
Maximum	13.5382
95% Confidence Interval for Mu	
9.8961	11.3107
95% Confidence Interval for Sigma	
1.3018	2.3496
95% Confidence Interval for Median	
9.2615	11.7757

Figure 29:descriptive statistics for concentrations of Advil® in plasma samples for the 24 subjects after 1.5hr.

2.9.2 Regression Analysis:

The regression equation is
Peak2 Hight. (mAu) = 4.20 + 0.687 St,d2 Conc.(ppm)

Predictor	Coef	StDev	T	P
Constant	4.199	3.858	1.09	0.308
St,d2 Con	0.68747	0.01083	63.47	0.000

S = 10.60 R-Sq = 99.8% R-Sq (adj) = 99.8%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	452209	452209	4028.08	0.000
Error	8	898	112		
Total	9	453107			

Unusual Observations

Obs	St,d2 Conc.	Peak2 Hig	Fit	StDev Fit	Residual	St.Resid
1	1000	678.62	691.67	9.53	-13.05	-2.81RX
2	500	373.12	347.93	4.85	25.19	2.67R

R denotes an observation with a large standardized residual

X denotes an observation whose X value gives it large influence

The concentration of Ibuprofen for any sample =[(Peak2 Height - 4.2) / 0.687].

In this case and upon calculating concentration of some samples it was found to be negative, which is unacceptable logically and upon omitting the extreme values of the standard curve the best fitted regression line was as seen bellow in Figure (30).

The regression equation is:

Peak1 Height (mAu) = - 0.168 + 0.773 St, d1 Conc. (ppm)

Predictor	Coef	StDev	T	P
Constant	-0.1678	0.2687	-0.62	0.555
St,d Con	0.772973	0.005533	139.71	0.000

S = 0.5532 R-Sq = 100.0% R-Sq(adj) = 100.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	5974.0	5974.0	19519.50	0.000
Error	6	1.8	0.3		
Total	7	5975.8			

Regression:

The regression equation is:

y = - 0.168 + 0.773 x

Predictor	Coef	StDev	T	P
Constant	-0.1678	0.2687	-0.62	0.555
x	0.772973	0.005533	139.71	0.000

S = 0.5532 R-Sq = 100.0% R-Sq(adj) = 100.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	5974.0	5974.0	19519.50	0.000
Error	6	1.8	0.3		
Total	7	5975.8			

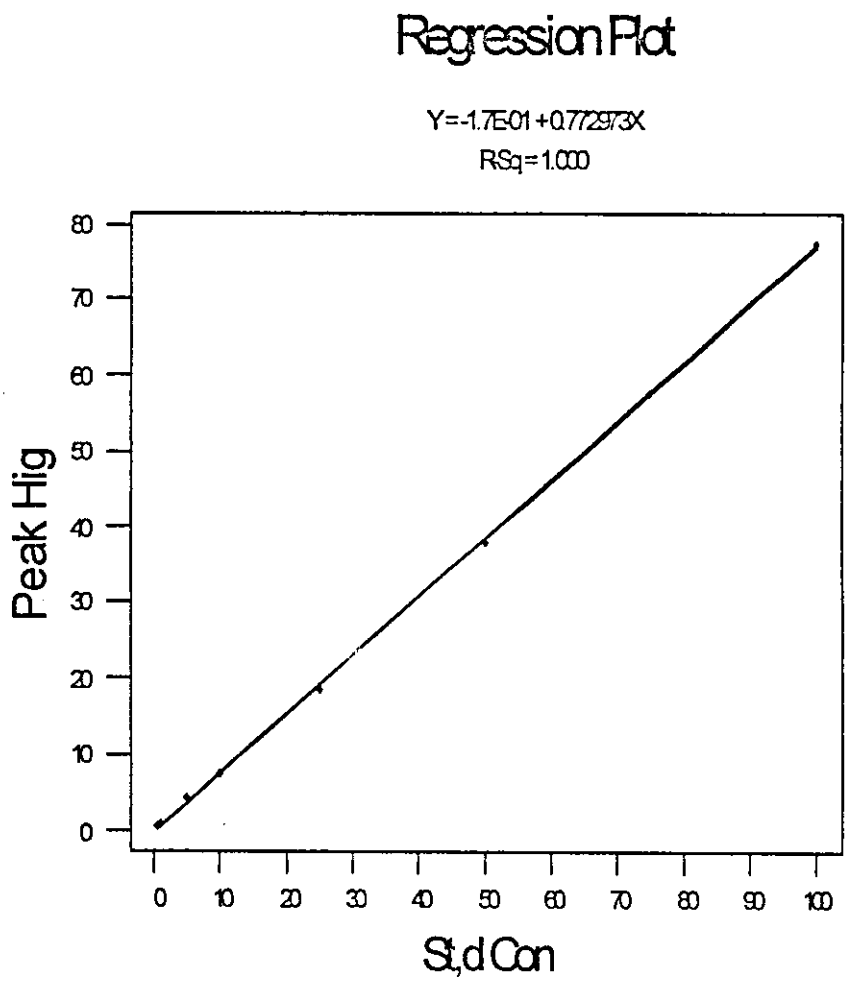


Figure 30:The best fitted regression line.

2.9.3 Analysis of variation:

1.One-Way Analysis of Variance (ANOVA test 1)

```
Analysis of Variance
Source     DF        SS        MS        F        P
Factor      2   196.144    98.072   237.13    0.000
Error      69    28.537     0.414
Total      71   224.681

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev ---+---------+---------+---------+---
C1*        24    4.7213    0.8241                                 (-*--)
C1         24    1.1382    0.1982 (-*--)
C1**       24    4.5514    0.7227                                 (-*-)
                                  ---+---------+---------+---------+---
Pooled StDev =    0.6431           1.2       2.4       3.6       4.8
```

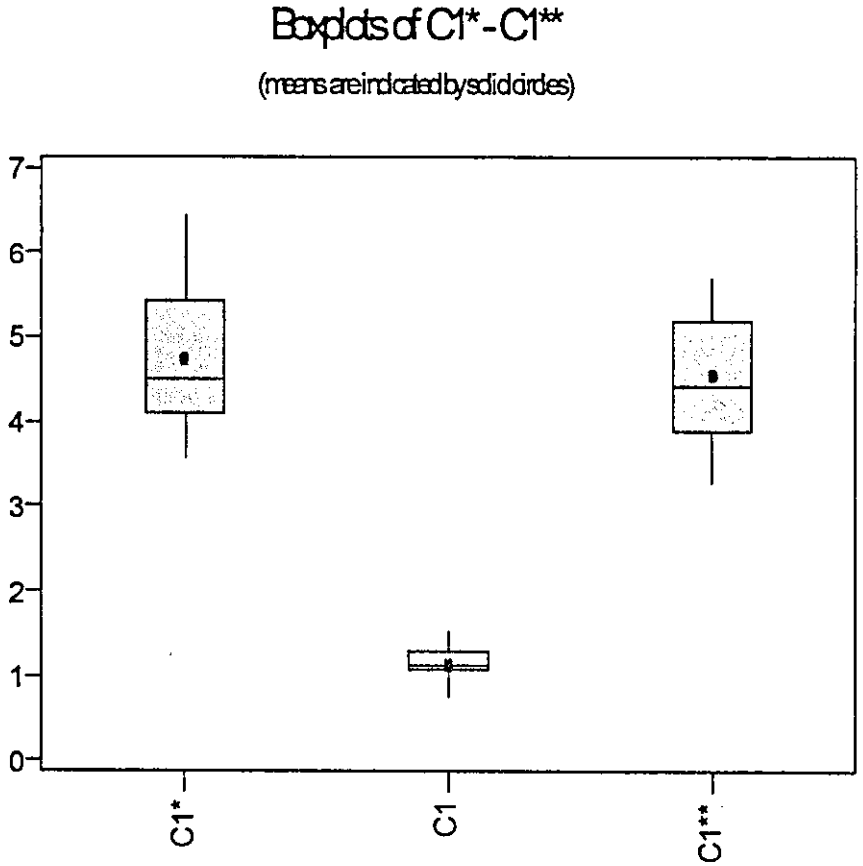


Figure 31: Boxplots of C1*, C1 and C1.**

2.One-Way Analysis of Variance (ANOVA test 2)

```
Analysis of Variance
Source      DF         SS         MS         F         P
Factor       2    462.618    231.309    275.32     0.000
Error       69     57.970      0.840
Total       71    520.588

                                        Individual 95% CIs For Mean
                                        Based on Pooled StDev
Level       N       Mean      StDev     ----+---------+---------+---------+--
C2*        24     7.1748     1.1343                                  (-*-)
C2         24     1.6897     0.2035    (*-)
C2**       24     6.9520     1.0920                                (-*-)
                                        ----+---------+---------+---------+--
Pooled StDev =     0.9166                 2.0       4.0       6.0       8.0
```

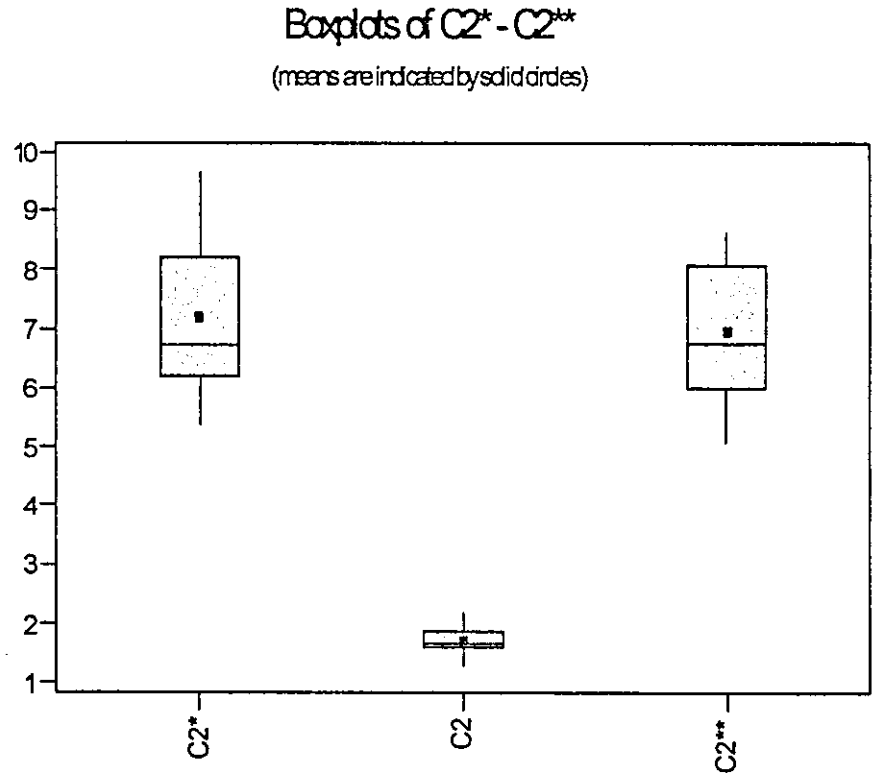


Figure 32: Box plots of C2*, C2 andC2.**

3.One-Way Analysis of Variance (ANOVA test 3)

```
Analysis of Variance
Source      DF        SS        MS        F        P
Factor       2   1056.36    528.18   252.77    0.000
Error       69    144.18      2.09
Total       71   1200.53

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev ---+---------+---------+---------+---
C3*        24    11.146     1.802                                 (-*-)
C3         24     2.763     0.465 (-*-)
C3**       24    10.603     1.675                           (-*-)
                                  ---+---------+---------+---------+---
Pooled StDev =    1.446            3.0       6.0       9.0      12.0
```

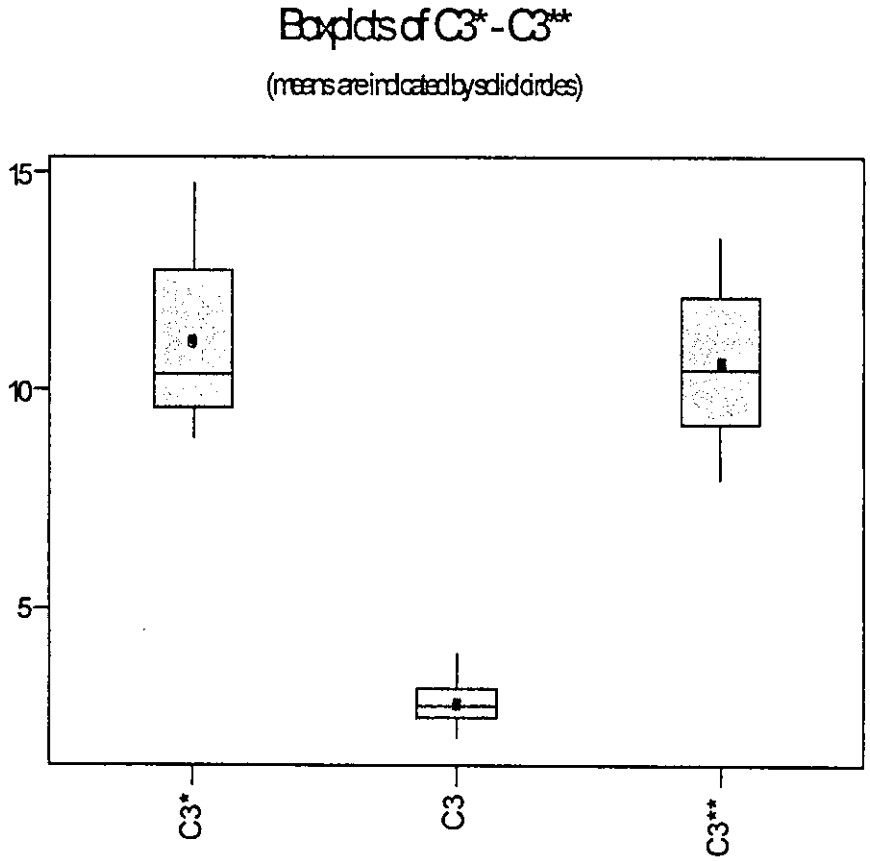


Figure 33:Boxplots of C3*, C3 and C3.**

2.9.4 Two Sample T- Tests

1. Two Sample T-Test (1) and Confidence Interval

Two sample T for C1* vs C1

	N	Mean	StDev	SE Mean
C1*	24	4.721	0.824	0.17
C1	24	1.138	0.198	0.040

95% CI for mu C1* - mu C1: (3.23, 3.931)
T-Test mu C1* = mu C1 (vs >): T= 20.71 P=0.0000 DF= 46
Both use Pooled StDev = 0.599

2. Two Sample T-Test (2) and Confidence Interval

Two sample T for C2* vs C2

	N	Mean	StDev	SE Mean
C2*	24	7.17	1.13	0.23
C2	24	1.690	0.204	0.042

95% CI for mu C2* - mu C2: (5.01, 5.959)
T-Test mu C2* = mu C2 (vs >): T= 23.32 P=0.0000 DF= 46
Both use Pooled StDev = 0.815

3.Two Sample T-Test (3) and Confidence Interval

Two sample T for C3* vs C3

	N	Mean	StDev	SE Mean
C3*	24	11.15	1.80	0.37
C3	24	2.763	0.465	0.095

95% CI for mu C3* - mu C3: (7.62, 9.148)
T-Test mu C3* = mu C3 (vs >): T= 22.07 P=0.0000 DF= 46
Both use Pooled StDev = 1.32

4.Two Sample T-Test (4) and Confidence Interval

Two sample T for C1** vs C1

	N	Mean	StDev	SE Mean
C1**	24	4.551	0.723	0.15
C1	24	1.138	0.198	0.040

95% CI for mu C1** - mu C1: (3.11, 3.721)
T-Test mu C1** = mu C1 (vs >): T= 22.31 P=0.0000 DF= 46
Both use Pooled StDev = 0.530

5.Two Sample T-Test (5) and Confidence Interval

Two sample T for C2** vs C2

	N	Mean	StDev	SE Mean
C2**	24	6.95	1.09	0.22
C2	24	1.690	0.204	0.042

95% CI for mu C2** - mu C2: (4.81, 5.719)
T-Test mu C2** = mu C2 (vs >): T= 23.21 P=0.0000 DF= 46
Both use Pooled StDev = 0.785

6.Two Sample T-Test (6) and Confidence Interval

Two sample T for C3** vs C3

	N	Mean	StDev	SE Mean
C3**	24	10.60	1.67	0.34
C3	24	2.763	0.465	0.095

95% CI for mu C3** - mu C3: (7.13, 8.555)
T-Test mu C3** = mu C3 (vs >): T= 22.09 P=0.0000 DF= 46
Both use Pooled StDev = 1.23

7.Two Sample T-Test (7) and Confidence Interval

Two sample T for C1* vs C1**

	N	Mean	StDev	SE Mean
C1*	24	4.721	0.824	0.17
C1**	24	4.551	0.723	0.15

95% CI for mu C1* - mu C1**: (-0.28, 0.62)
T-Test mu C1* = mu C1** (vs >): T= 0.76 P=0.23 DF= 46
Both use Pooled StDev = 0.775

8.Two Sample T-Test (8) and Confidence Interval

Two sample T for C2* vs C2**

	N	Mean	StDev	SE Mean
C2*	24	7.17	1.13	0.23
C2**	24	6.95	1.09	0.22

95% CI for mu C2* - mu C2**: (-0.42, 0.87)
T-Test mu C2* = mu C2** (vs >): T= 0.69 P=0.25 DF= 46
Both use Pooled StDev = 1.11

9.Two Sample T-Test (9) and Confidence Interval

Two sample T for C3* vs C3**

	N	Mean	StDev	SE Mean
C3*	24	11.15	1.80	0.37
C3**	24	10.60	1.67	0.34

95% CI for mu C3* - mu C3**: (-0.47, 1.55)
T-Test mu C3* = mu C3** (vs >): T= 1.08 P=0.14 DF= 46
Both use Pooled StDev = 1.74

10.Two Sample T-Test(10) and Confidence Interval

Two sample T for C1M* vs C1F*

	N	Mean	StDev	SE Mean
C1M*	12	4.850	0.904	0.26
C1F*	12	4.592	0.753	0.22

95% CI for mu C1M* - mu C1F*: (-0.45, 0.96)
T-Test mu C1M* = mu C1F* (vs not =): T= 0.76 P=0.46 DF=21

11.Two Sample T-Test(11) and Confidence Interval

Two sample T for C2M* vs C2F**

	N	Mean	StDev	SE Mean
C2M*	12	7.21	1.24	0.36
C2F**	12	7.12	1.03	0.30

95% CI for mu C2M* - mu C2F**: (-0.87, 1.06)
T-Test mu C2M* = mu C2F** (vs not =): T= 0.20 P=0.84 DF= 21

12.Two Sample T-Test(12) and Confidence Interval

Two sample T for C3M* vs C3F*

	N	Mean	StDev	SE Mean
C3M*	12	11.18	2.11	0.61
C3F*	12	11.12	1.53	0.44

95% CI for mu C3M* - mu C3F*: (-1.51, 1.63)
T-Test mu C3M* = mu C3F* (vs not =): T= 0.08 P=0.94 DF= 20

13.Two Sample T-Test(13) and Confidence Interval

Two sample T for C1M vs C1F

	N	Mean	StDev	SE Mean
C1M	12	1.148	0.234	0.068
C1F	12	1.129	0.165	0.048

95% CI for mu C1M - mu C1F: (-0.154, 0.192)
T-Test mu C1M = mu C1F (vs not =): T= 0.23 P=0.82 DF= 19

14.Two Sample T-Test(14) and Confidence Interval

Two sample T for C2M vs C2F

	N	Mean	StDev	SE Mean
C2M	12	1.738	0.264	0.076
C2F	12	1.641	0.109	0.032

95% CI for mu C2M - mu C2F: (-0.080, 0.273)
T-Test mu C2M = mu C2F (vs not =): T= 1.17 P=0.26 DF= 14

15.Two Sample T-Test(15) and Confidence Interval

Two sample T for C3M vs C3F

	N	Mean	StDev	SE Mean
C3M	12	2.818	0.338	0.097
C3F	12	2.708	0.576	0.17

95% CI for mu C3M - mu C3F: (-0.296, 0.52)
T-Test mu C3M = mu C3F (vs not =): T= 0.57 P=0.57 DF= 17

16.Two Sample T-Test(16) and Confidence Interval

Two sample T for C1M** vs C1F**

	N	Mean	StDev	SE Mean
C1M**	12	4.433	0.767	0.22
C1F**	12	4.669	0.688	0.20

95% CI for mu C1M** - mu C1F**: (-0.85, 0.38)
T-Test mu C1M** = mu C1F** (vs not =): T= -0.79 P=0.44
DF= 21

17.Two Sample T-Test(17) and Confidence Interval

Two sample T for C2M** vs C2F**

	N	Mean	StDev	SE Mean
C2M**	12	6.79	1.17	0.34
C2F**	12	7.12	1.03	0.30

95% CI for mu C2M** - mu C2F**: (-1.27, 0.61)
T-Test mu C2M** = mu C2F** (vs not =): T= -0.74 P=0.47
DF= 21

18.Two Sample T-Test(18) and Confidence Interval

Two sample T for C3M** vs C3F**

	N	Mean	StDev	SE Mean
C3M**	12	10.32	1.80	0.52
C3F**	12	10.89	1.57	0.45

95% CI for mu C3M** - mu C3F**: (-2.00, 0.87)
T-Test mu C3M** = mu C3F** (vs not =): T= -0.82
P=0.42 DF= 21

2.9.5 One Sample T-tests:

1.One sample T-Test (1) of the Mean

Test of mu = 10.000 vs mu > 10.000

Variable	N	Mean	StDev	SE Mean	T	P
C3*	24	11.146	1.802	0.368	3.12	0.0024

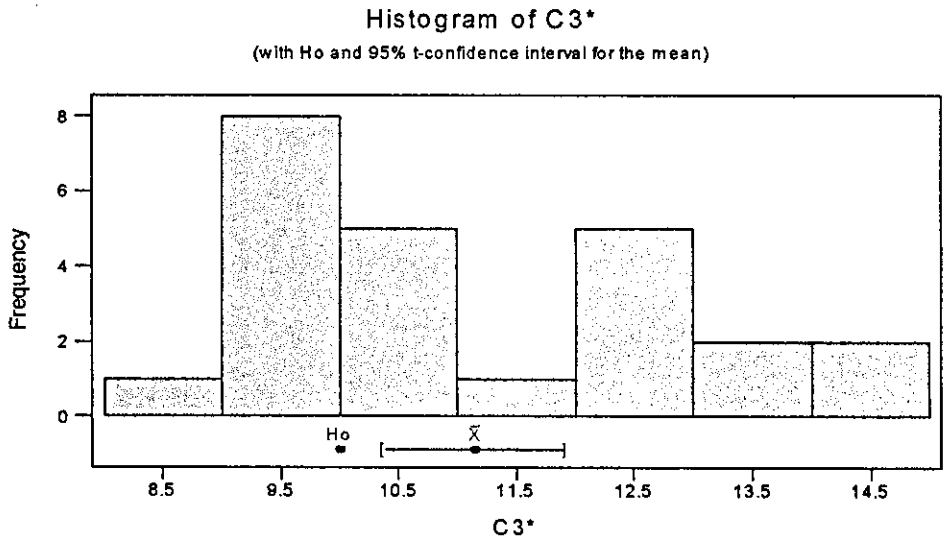


Figure 34: Histogram of C3*.

2.One sample T-Test(2) of the Mean

Test of mu = 10.0000 vs mu > 10.0000

Variable	N	Mean	StDev	SE Mean	T	P
C3	24	2.7630	0.4653	0.0950	-76.19	1.00

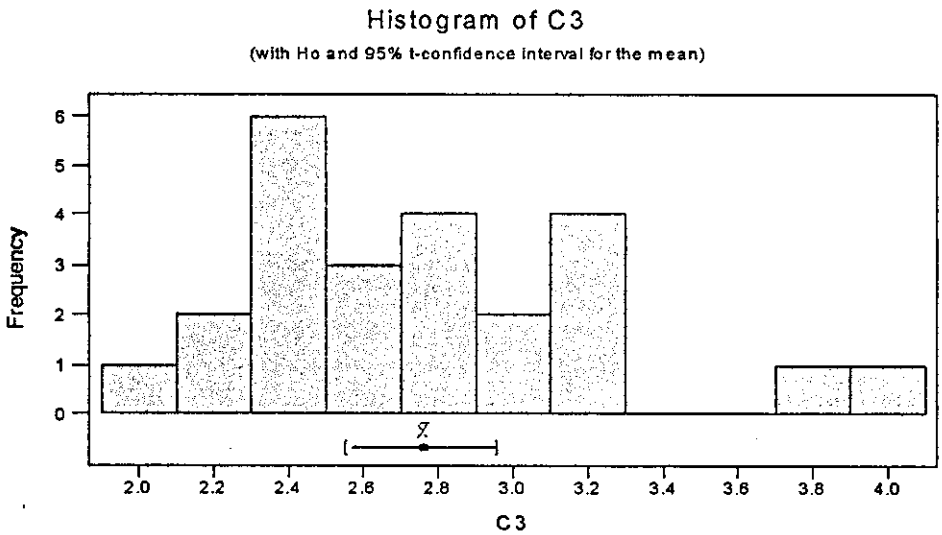


Figure 35: Histogram of C3.

3. One sample T-Test(3) of the Mean

Test of mu = 10.000 vs mu > 10.000

Variable	N	Mean	StDev	SE Mean	T	P
C3**	24	10.603	1.675	0.342	1.76	0.045

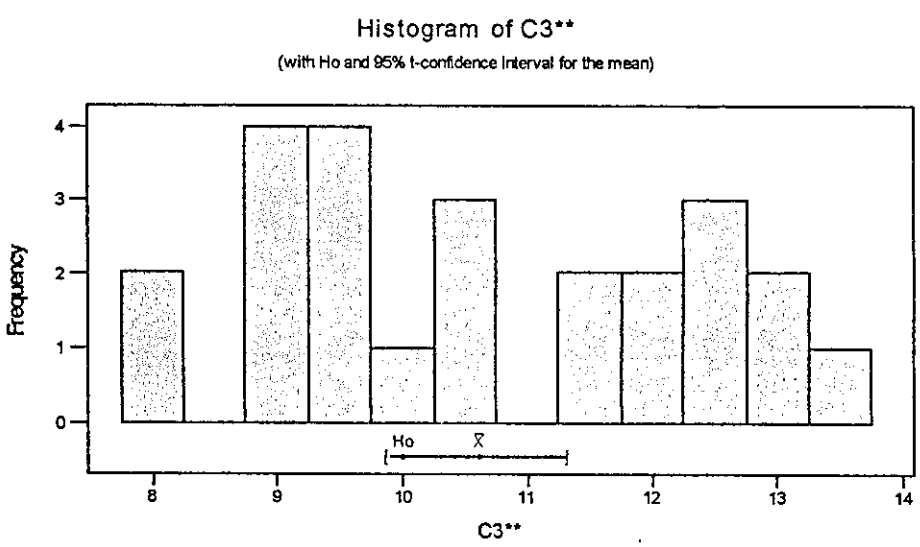


Figure 36: Histogram of C3.**

For the active ingredient assay the chromatogram is shown in the figure (37) below.

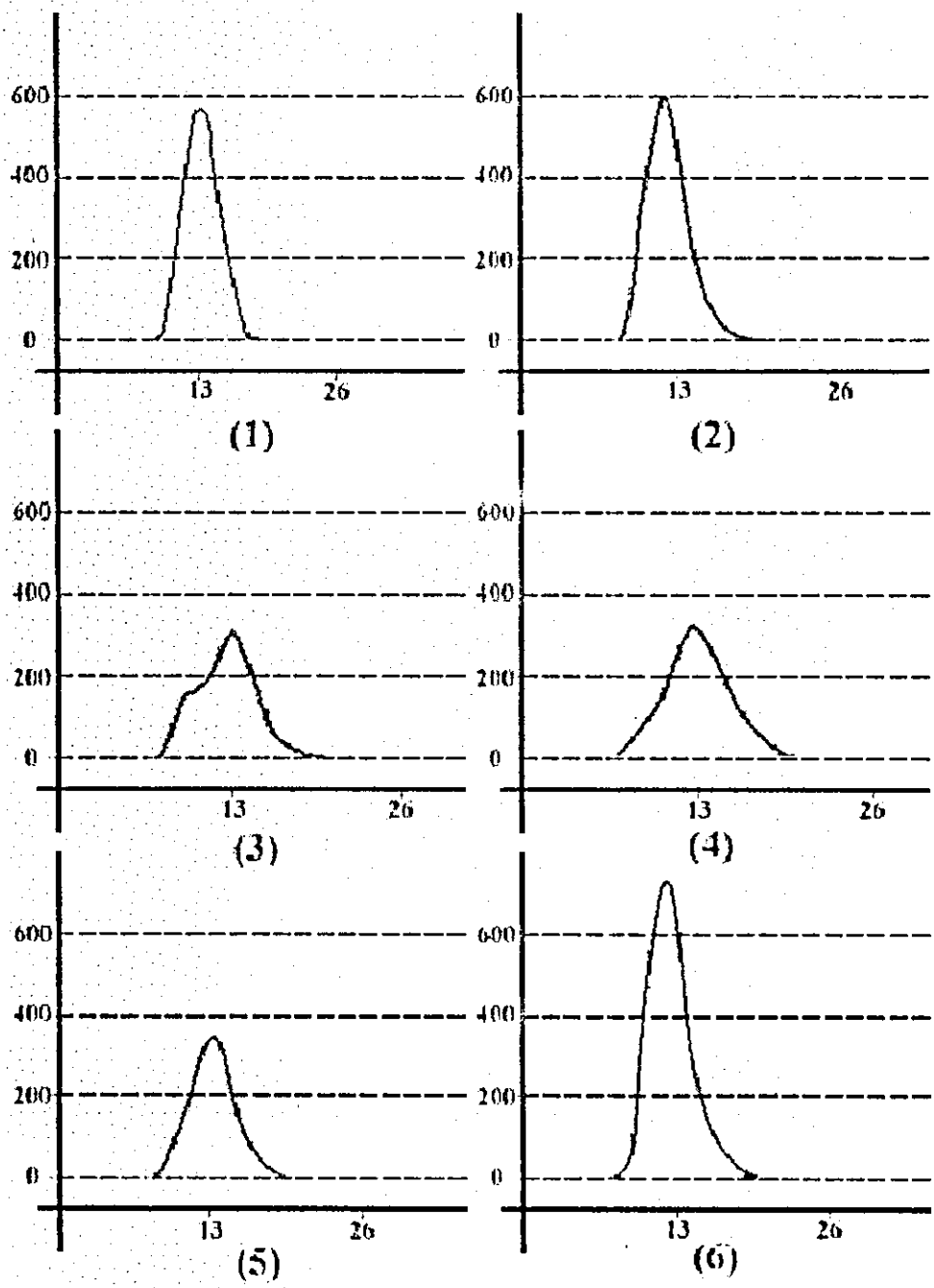


Figure 37: Chromatogram for Ibuprofen active ingredient assay.
Note: 1 for Nurofen® (1000 ppm, 568.2 mAu), 2 for Local brand® (1000 ppm, 606.2 mAu), 3 for Standard (500 ppm, 318 mAu), 4 for Nurofen® (500 ppm, 335 mAu), 5 for Local brand ® (500 ppm 365 mAu) and 6 for standard (1000 ppm, 702 mAu).

The stated % Ibuprofen (Wt/Wt) in the local brand tablets = (400/618.5)* 100% = 64.67 %.

It's clear that the quantity of Ibuprofen in the local brand tablets is closer to the stated amount than that of Nurofen®, but on the account of inactive ingredients, which I believe, have a joint role in the overall drug's life cycle in the body.

2.9.6.2 Dissolution test result:

Sample No.	Absorbance (Abs.U)	Sample absorbance/St,d absorbance
1	0.61182	0.9412
2	0.63099	0.9707
3	0.62082	0.9551
4	0.60408	0.9293
5	0.61128	0.9404
6	0.61398	0.9445

Table 9:Samples absorbances for dissolution test.

Correction for st' d weight = 0.579 Abs.U* 100/99 = 0.585.

Correction for st' d concentration = 0.585Abs.U*0.444/0.4 =0.65 Abs.U.

The sample passes the test since the quantity of each active dissolved from the unit tested conforms to the accompanying acceptance table (10). Testing was continued through the three stages unless the results conform at S1, S2 or S3.Tolerance–Not less than 80 %(Q) of Ibuprofen dissolved in 60 minutes.

Stage	No. Tested	Acceptable Criteria
S1	6	Each unit is not less than Q+5%
S2	6	Average of 12 units (S1 +S2) is equal to or greater than Q, and no unit is less than Q- 15%
S3	12	Average of 24 units (S1+S2+S3) is equal to or greater than Q, not more than 2 units are less than Q – 15%, and no unit is less than Q – 25%.

Table (10): Accompanying acceptable table

Although the sample passes the test, this doesn't mean that the *in vitro* dissolution test is the only determinating factor in the final overall drug bioavailability, but it,s indicativity to some extent depending upon the degree of correlation between the *in vivo* and *in vitro* dissolution, since there are also a lot of

factors that affect the *in vivo* dissolution of the drug other than that of the *in vitro*.

It is clear from the analysis of variations (ANOVA tests 1, 2 and 3) that there is a significant difference (at $\alpha = 0.05$) between the concentrations of the three drugs in plasma samples at the three periods of time 0.5 hr, 1 hr and 1.5 hr and this subsequently means that there are at least two drugs that are different in their rates of absorption, but not by necessity in their bioavailabilities.

However to examine which two drugs are significantly different multiple comparisons between the concentrations of each pair of drugs in plasma at the three periods of time have to be done.

It was clarified after carrying out the above comparisons in T- tests from 1 to 9 that:

1. In T-tests from 1 to 6 it was found with a high degree of validation that the means of concentrations of the Nurofen® and Advil® drugs are higher than that of the local brand at the three periods of time which means

that their rates of absorption are higher than that of the local brand.

2. In T-tests from 7 to 9 it was found that there is no significant difference between the means of Nurofen® and Advil® concentrations at the three periods of time which means that their rates of absorption are almost equal.

3. In two sample T-tests from 10 to 18 it was found that there is no significant difference in blood plasma concentration between males and females at the three periods of time.

4. In one sample T-tests (1 & 3) It's obvious that the mean concentrations of both Nurofen® and Advil® after the hypothesized T_{max} (1.5 hr) are within the therapeutic range (10-50 ppm) although the two means are low, but the mean of the local brand concentration is bellow the minimum effective concentration which may be responsible partially for the lack of therapeutic efficacy that results when a local brand is used in the treatment.

CHAPTER THREE

DISCUSSION

& RECOMMENDATIONS

3. DISCUSSION & RECOMMENDATIONS:

3.1 Causes of Low Bioavailability:

The entry of a drug into the systemic circulation following the administration of the drug product usually involves:

1. The release of the drug from its dosage form into solution in the biological fluids at the absorption site, and

2. The movement of the dissolved drug across biological membranes into the systemic circulation and these two processes may vary in their rates from one pharmaceutical equivalent to another as had been shown in the case of the local brand relative to the other pharmaceutical equivalents (Nurofen® & Advil®).

Dressman *et al.* (Dressman, 1998) list the following physicochemical and physiological parameters as important determinants of *in vivo* drug dissolution:

❖ Surface area:

1. Particle size and wettability

2. Surfactants in gastric juice

3. Bile salts

❖ Diffusivity:

1. Molecular size

2. Viscosity of lumenal contents

❖ Boundary layer thickness:

1. Gastrointestinal motility pattern

2. Fluid flow rate

❖ Solubility:

1. Hydrophilicity

2. Crystalline structure,

3. Gastrointestinal pH and buffering capacity

4. Bile

5. Food components

❖ Maintenance of sink conditions:

1. Gastrointestinal fluid flow

2. Drug permeability

❖ Volume of solvent available:

1. Secretions

2. Co-administered fluids

The impact of these factors must be considered when developing *in vitro* methods for predicting product quality and *in vivo* performance. However, in some cases, it is not drug solubility or product dissolution that limits bioavailability but rather it is the ability of the drug to cross the gastrointestinal (GI) mucosa. Depending upon what constitutes the rate-limiting step in the drug absorption process, physiological interindividual differences, whether due to differences in GI transit time, GI fluid composition, site of absorption, disease, etc., can markedly impact *in vivo* dissolution and thus drug bioavailability. Therefore, a pivotal question is whether or not it is possible to identify those compounds and *in vitro* test conditions that can assure product bioequivalence across a range of physiological states (Dressman, 1998).

In our study case we believe that we can exclude these interindividual physiological differences because if they ever play a role, their effect will be similar in the three pharmaceutical equivalents (Local brand, Nurofen® and Advil®).

The *in vitro in vivo* correlation (IVIVC) enables a dissolution test to be used as a surrogate of the bioavailability study, it basically relates the amount of drug dissolved in vitro to the amount of drug absorbed *in vivo* using appropriate mathematical functions and suitable dissolution test conditions.

The in *vivo* drug performance then is predicted based on the correlation function as well as dissolution parameters (Rockville, 1997).

One of the most significant prognostic tools developed in recent years has been the (BCS) (Amidon, 1995). By knowing a compound's solubility and intestinal permeability characteristics, drugs are classified into one of four categories:

* Class I:

High solubility, high permeability. These compounds are generally very well absorbed. Examples include propranol and metoprolol. For those Class I compounds formulated as immediate release products, dissolution rate generally exceeds gastric emptying. Therefore, nearly 100% absorption can be expected if at least 85% of a product

dissolves within 30 min of *in vitro* dissolution testing across a range of pH values (Food &Drug Administration, 8/2000; Food &Drug Administration, 10/2000). Accordingly, *in vivo* bioequivalence data are not necessary to assure product comparability.

* Class II:

Low solubility, high permeability. The bioavailability of products containing these compounds is likely to be dissolution-rate limited. For this reason, a correlation between *in vivo* bioavailability and *in vitro* dissolution rate an (IVIVC) may be observed, but it is not a point-to-point correlation. Examples include piroxicam and naproxen. I believe that Ibuprofen is located within this class of drugs, and so the *in vitro* dissolution isn't the final determinating factor in the overall drug's bioavailability.

* Class III:

High solubility, low permeability: Absorption is permeability-rate limited but dissolution will most likely occur very rapidly.

For this reason, there has been some suggestion that as long as the test and reference formulations do not contain

agents that can modify drug permeability or GI transit time, waiver criteria similar to those associated with Class I compounds may be approppriate. Examples include ranitidine and cimetidine.

* Class IV:

Low solubility, low permeability: very poor oral bioavailability. These compounds are not only difficult to dissolve but once dissolved, often exhibit limited permeability across the GI mucosa. These drugs tend to be very difficult to formulate and can exhibit very large intersubject and intrasubject variability. Examples of these compounds include furosemide and hydrochlorothiazide.

3.2 Formulation and manufacturing effects:

3.2.1 Formulation Effects:

It may be helpful to review the types of excipients that might be included in an oral dosage form and their function. A brief overview is provided bellow.

It should be noted that lubricants and glidants must be added in very small quantities and should be subjected to minimal blending times. These hydrophobic compounds can impede drug dissolution by coating drug particles, thereby

decreasing the area of drug–solvent interface and reducing particle wettability (Abdou, 1989). Additionally, certain compounds that are administered in small quantities, such as cardiac glycosides, alkaloids, synthetic estrogens and steroids, can be adsorbed to the surface of some diluents and disintegrants, thereby lowering their bioavailability (Ansel, 1999).

1. Filler:

A filler (diluent) is often needed to increase the bulk of a formulation when the amount of drug substance is insufficient to produce a tablet of practical size. Examples include lactose, dicalcium phosphate and pregelatinized starch.

2. Disintegrant:

A substance routinely included in tablet formulations (and many hard shell capsule formulations) to promote moisture penetration and dispersion of the matrix of the dosage form in dissolution fluids to expose primary drug particles. Through swelling or other mechanisms, disintegrants overcome the cohesive strength introduced into the mass by compression and by any binder present.

Examples include starch, sodium starch glycolate, croscarmellose, and crospovidone.

3. Lubricants:

Lubricants are substances that (1) act to reduce friction at the die wall during tablet compression and ejection (the 'true lubricant' role), (2) reduce adhesion to punch faces (the 'antiadherent' role, and (3) promote powder flow by reducing interparticle friction and cohesion (the 'glidant' role). Lubricants generally are not equally efficient at all three roles. For example, colloidal silicon dioxide, often considered an 'excellent' glidant, can provide 'good' antiadherency, but it is not effective in reducing friction at the die wall. Magnesium and calcium stearates are considered 'excellent' true lubricants and 'good' antiadherents, but are less effective as glidants.

4. **Binders:**

Binders are adhesives added to wet-granulate powders. Whether added as a binder solution or dry blended with the powders followed by wetting with a solvent, the binder serves as a 'glue' that facilitates the agglomeration and adhesion of the particles into granules. When dried and

sized, granules flow better than the original powder. When the granules are compressed into a tablet, the binder helps hold the tablet together. Examples include:

Polyvinylpyrrolidone (PVP), hydroxypropylmethylcellulose (HPMC), and pregelatinized starch.

5. Filler binders:

These special fillers make tableting of many low-to-moderate dose drugs by direct compression practical. They may be selected physical forms of conventional fillers that flow and/or compact well (e.g. microcrystalline cellulose, unmilled dicalcium phosphate dihydrate) or fillers that have been physically modified to give them these qualities (e.g. spray processed lactose).

6. Surfactans:

A surfactant may be included in a formulation to increase the wetting of a powder mass or tablet matrix and enhance dissolution of the drug. Examples include sodium lauryl sulfate and sodium docusate.

7. Antioxidants:

Antioxidants such as ascorbyl palmitate may be included in a formulation to provide chemical stability by inhibiting oxidation.

8. Coating agents:

Commonly, polymer-based films are applied to modern tablets for multiple reasons, such as to provide protection from atmosphere, improve aesthetics, or modify drug release (sustained / controlled release or delayed release). Enteric coatings can protect sensitive drugs from inactivation in gastric fluid by delaying release until the dosage passes to the intestine. Examples include HPMC, ethyl cellulose latexes, HPMC phthalate (enteric), and polymers and esters of methacrylic acid (enteric and sustained release functions).

It was clear from the active ingredient assay that the percentage of the inactive ingredients in Trufen® are higher than that of Nurofen® and Advil® and these excepients as we have just shown play an important role in the final bioavailability of the drug and this role is of qualitative and quantitative importance.

3.2.2 Manufacturing Effects:

The active ingredient and final product manufacturing includes a lot of processes such as crystallization, precipitation, filtration, emulsification, milling, mixing, drying, granulation, tabletting, compression, autoclaving, handling, storage and transportation and these processes vary in the rate and extent from one formulation to another.

For example the selection of tableting process is a critical variable in the optimization of drug product bioavailability. The three most common methods include (Ansel, 1999):

1. Wet granulation: improves the dissolution rates of poorly soluble drugs by imparting hydrophilic properties to the surface of the granules.

2. Dry granulation: used when the drug is sensitive to moisture or heat.

3. Direct compression: the most cost effective of the three manufacturing methods, it can be used when the drug and excipients are free flowing, cohesive and do not degrade under the heat and pressure associated with the tableting process.

3.2.3 Particle characteristics:

3.2.3.1 Shape:

The crystalline form of the drug can markedly affect its solubility. About one third of all organic drug substances are polymorphic, including crystalline versus non-crystalline (amorphous) forms (Ansel, 1999).

Each polymorph usually exhibits its own distinct physicochemical properties, including melting point and aqueous solubility. In fact, amorphous powders are almost always the more water-soluble of the crystalline forms since they require less energy for dissolution. This in turn can affect bioavailability. For example in the case of Ibuprofen, if crystallization is carried out in the presence of additives, special effects can occur that influence the crystal habit and the crystal surface.

Thus the properties of the resulting drug powder can be affected, even if a pure drug results. Effects that are not obtainable by different crystallization processes without additives can be achieved. In Ibuprofen crystals the hydrogen bonds play an important role. Additives that are able to interact strongly with the hydrogen bonds of

ibuprofen are able to affect the growth rate of the dominant (100) surface transferring the growth rate to the polar variant of the surface. Especially the carboxylic group of sodium salts of fatty acids or the sucrose esters (many hydroxyl-groups) are suitable for this strong interaction. Geometrically exactly shaped crystals are formed, which shows the influence on the crystallization process. The environment affects the external shape of the Ibuprofen crystal, without changing the internal structure; all crystals were isomorphic. By the use of additives during the crystallization process, an improvement of handling properties and of dissolution properties can be reached see figures (38,39) bellow (Norbert Rasenack, 2002).

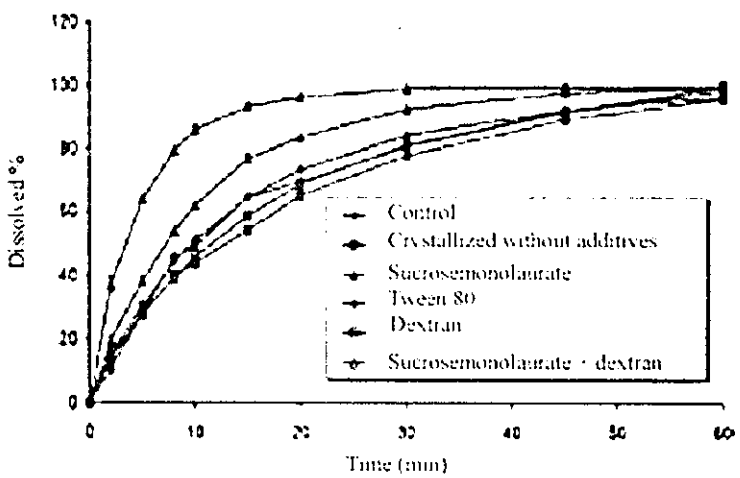


Figure 38: effects of additives in crystallization process on dissolution % of Ibuprofen.

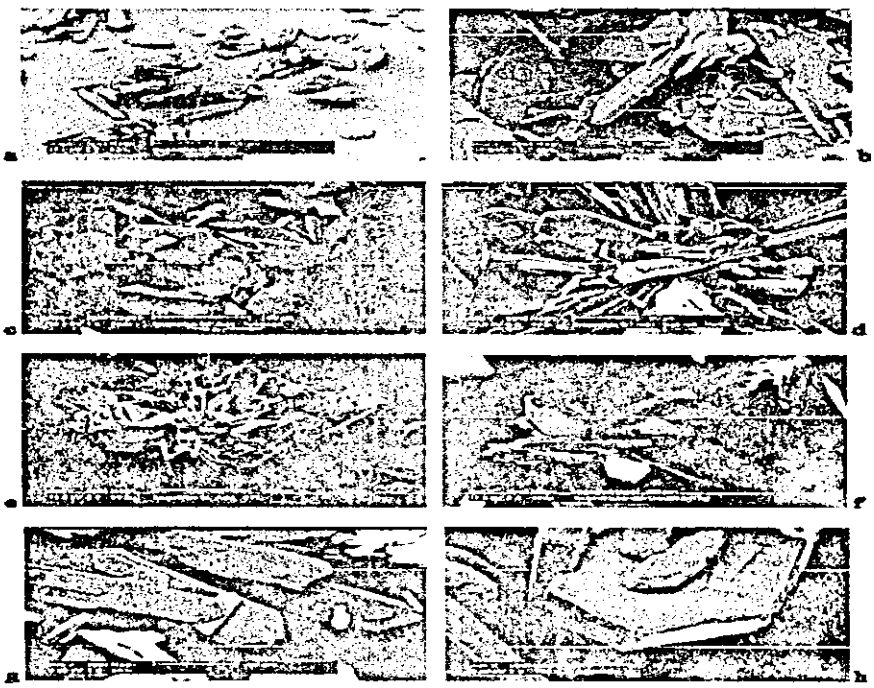


Figure 39: photographs of the ibuprofen crystals. (a) Control; (b) crystallized without additives; (c) crystallized in the presence of polysorbate 80; (d) crystallized in the presence of sucrosemonolaurate; (e) crystallized in the presence of hydroxypropyl cellulose; (f) crystallized in the presence of sucrosemonolaurate and dextran 200; (g, h) crystallized in the presence of sucrosemonolaurate and hydroxypropyl cellulose.

So it may be there is a diference between the imported polymorphic Ibuprofen quality for use in the local pharmaceutical industry and that used in the foreign industries.

Formulators often face a double-edged sword when attempting to improve product bioavailability. On the one hand, the drug must possess sufficient lipophilicity to ensure its permeability across biological membranes. On the other

hand, the drug must have sufficient hydrophilicity to enable it to dissolve in GI fluids. One method of addressing this issue is through the use of excipients that either promote dissolution of highly lipophilic compounds (e.g. the inclusion of surfactants) or penetration enhancers that increase the membrane permeability of hydrophilic molecules. Another option is the modification of the active ingredient by the use of covalently linked complexes, such as ionic or inclusion dissolution complexes (Sabnis, 1999) and those may vary in quantity and quality between the current study pharmaceutical equivalents that leaded subsequently to serious bioequivalence problems.

Certain excipients may alter *in vivo* dissolution without affecting *in vitro* dissolution due to their effect on GI transit time. For example, owing to its osmotic activity, certain sugar alcohols (such as mannitol) decrease GI transit time, resulting in more rapid dosage form transit through the intestine, but sucrose is without effect on GI transit time (Adkin, 1995).

Other excipients shown to hasten small intestinal transit (and therefore decrease the bioavailability of low

permeability compounds) include sodium acid pyrophosphate (Adkin, 1995). These effects are known to occur at concentrations relevant to pharmaceutical formulations.

3.2.3.2 Size:

Both size and density can modify in vivo particle dispersion, with greater dispersion resulting in improved dissolution of poorly soluble and slowly dissolving drugs (Gupta, 1995). This consideration may be particularly important when formulating Class II compounds or sustained release preparations. Since hydrophilic polymers can facilitate particle dispersion via increasing fluid viscosity, both viscosity and particle characteristics (e.g. shape, size and density) may be critical formulation variables impacting cross-species differences in product bioavailability (Gupta, 1995).

Modified release microspheres of the NSAID, Ibuprofen, were formulated and prepared using the emulsion solvent diffusion technique. The contribution of various dispersed phase and continuous phase formulation factors on *in vitro* drug release and micromeritic characteristics of

microspheres was examined. The results demonstrated that the use of Eudragit RS 100 and Eudragit RL 100 as embedding polymers modified the drug release properties as a function of polymer type and concentration (Perumal, 1999). Eudragit RS 100 retarded Ibuprofen release from the microspheres to a greater extent than Eudragit RL 100. The drug/polymer concentration of the dispersed phase influenced the particle size and drug release properties of the formed microspheres. It was found that the presence of emulsifier was essential for microsphere formation (Perumal, 1999).

Increasing the concentration of emulsifier, sucrose fatty acid ester F-70, decreased the particle size, which contributed to increased drug release properties. Scanning electron microscopy revealed profound distortion in both the shape and surface morphology of the microspheres with the use of magnesium stearate as added emulsifier.

The application of an additional Eudragit RS 100 coat onto formed microspheres using fluid bed technology was successful and modulated the drug release properties of the coated microspheres (Perumal, 1999), so employing such

micronization technologies and drug's releasing ability enhancers may improve the biological performance of the local brand.

3.3 Recommendations:

❖ Enactment of strict regulations belonging to drug quality control with full commitment in its application.

❖ Enhancement of cooperative and integrative liaison between institutional, academic and eligible governmental laboratories in the context of drug bioavailability optimization for the sake of protecting the public health.

❖ Maximizing drug bioavailability at the drug design stage by the formulating companies via following up new inventions in the pharmaceutical industry as well applying mathematical modeling technique.

❖ Performing more pharmacokinetic studies on the medications available in the local market.

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بسم الله الرحمن الرحيم

الموضوع : الموافقة على المشاركة في دراسة التوافر الحيوي لدواء Ibuprofen

أنا الموقع أدناه ..... أوافق بمحض إرادتي على المشاركة في دراسة التوافر الحيوي لمادة Ibuprofen علما بأنني تناولت هذا الدواء في الماضي دون حدوث أي مضاعفات..وقد تم توضيح جميع ما يتعلق بهذه التجربة لي قبل قيامي بالمشاركة. وأنا أخلي الجامعة وكوادرها من أي مسؤولية قانونية مترتبة على هذه الدراسة.

الاسم :

التاريخ :

التوقيع :

" إستبانة Questionnaire "

- هل سبق أن تناولت Ibuprofen في السابق؟

أ- نعم ب- لا (إذا أجبت بلا فالرجاء عدم المشاركة)

- أذكر أي مضاعفات (إن وجدت) حصلت لك عند تناولك عقار Ibuprofen من المضاعفات المذكورة أدناه :

أ- طفح جلدي

ب- صفير في الصدر أو أزمة أو لحميات أنفية

ج- ازدياد أعراض الأزمة (إذا كان لديك أزمة)

د- آلام في البطن

هـ- نزيف في أي مكان في الجسم

و- فشل كلوي

ز- غير ذلك : الرجاء التحديد .....

- العمر : .....

- الوزن : .....

- الطول : .....

جامعة النجاح الوطنية
كلية الدراسات العليا

مقارنة التوافر الحيوي لثلاث تركيبات علاجية متكافئة لدواء الأيبوبروفين (نوعية محلية، نيوروفين إنجليزي والأدفيل الأمريكي) في السوق المحلي

إعداد

عبد الرحمن محمد أمين جرار

إشراف

د. أنسام صوالحة

قدّمت هذه الرسالة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم البيئية بكلية الدراسات العليا في جامعة النجاح الوطنية.

نابلس – فلسطين

تموز 2003م

بسم الله الرحمن الرحيم

ملخص

يهدف هذا البحث الى مقارنة التوافر الحيوي لثلاث تركيبات علاجية متكافئة لدواء الأيبوبروفين احداها ذو صناعة محلية والآخرين صناعة اجنبية وهما النيوروفين والأدفيل.من اجل القيام بهذه الدراسة كان لا بد من اخذ ثلاث عينات من الدم على الأقل وعلى ثلاث فترات(نصف ساعة ، ساعة وساعةونصف) وذلك بعد ان ياخذ كل شخص من كل مجموعة (ثلاث مجموعات وكل مجموعة تضم ٢٤ متبرع من طلبة الجامعة من كلا الجنسين) قرصا من احد الأدوية .تمت عملية اخذ العينات في المختبرات المركزية للجامعة ، كما تمت عملية تحليل العينات من اجل قياس تركيز الأيبوبروفين في كل عينة وذلك بواسطة جهاز (HPLC/U.V) . بعد ان تمت عملية التحليل الأحصائي للنتائج فقد تبين ان هناك فرقا ذا دلالة احصائية في معدل الامتصاص بين الأدوية الثلاثة حيث انه كان الأقل في حالة ألنوعية ألمحلية بينما كان متساويافي حالة النيوروفين والأدفيل، كما ان تركيز الأيبوبروفين في ألدم للنوعية المحلية كان دون المستوى العلاجي المطلوب.أرجو أن تكون هذه الدراسة قد ساهمت في مجال الرقابة الدوائية وذلك من اجل المحافظة على صحة مجتمعنا .