DETERMINATION OF ACTIVE COMPOUNDS IN ANALGESIC
FORMULATIONS USING HPLC

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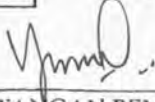4. **Sila tandakan (/)

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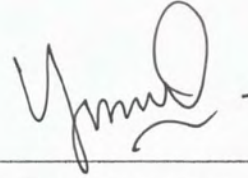@ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).

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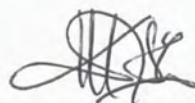
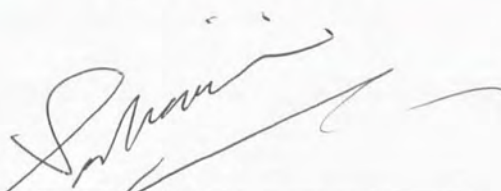MAY, 2008

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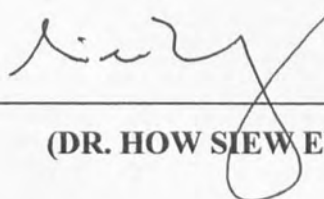
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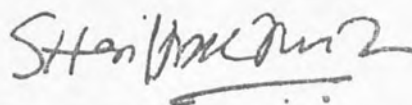HPLC

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ABSTRACT

Non-narcotic analgesics act peripherally by blocking pain signals going to the brain or by interfering with the brain's interpretation of the signals, without producing anesthesia or loss of consciousness. This study attempted to analyse the presence of phenacetin, caffeine and acetyl salicylic acid in nine types of different analgesic formulation (Panadol Normal, Panadol Actifast, Panadol Muscle and Joint, Panadol Kids, Panadol Soluble, Bayer Aspirin, Alka-Seltzer, Disprin and G-Asp Tablet) using a reversed phase High-Performance Liquid Chromatography (HPLC). Separation was completed in < 6 min. The result of the analysis showed that the Bayer Aspirin, Alka-Seltzer, Disprin and G-Asp Tablet contained acetyl salicylic acid as the active compound with concentration of 181.31 mg/g, 15.84 mg/g, 63.87 mg/g, and 10.11 mg/g, respectively. Among the 4 types of tablets, Bayer Aspirin had the strongest analgesic strength since it contained the most amount of acetyl salicylic acid.

ABSTRAK

PENENTUAN SEEBATIAN-SEBATIAN AKTIF DALAM RUMUSAN

ANALGESIK DENGAN MENGGUNAKAN KROMATOGRAFI CECAIR

PRESTASI TINGGI

Analgesik bukan narkotik berfungsi secara periferi dengan menghalang penyampaian isyarat sakit ke otak atau dengan mengacau interpretasi isyarat oleh otak, tanpa menghasilkan anestesia atau hilang kesedaran. Kajian ini bertujuan untuk menganalisis kehadiran fenasetin, kafein dan asid salicilik asetil dalam sembilan jenis rumusan analgesik yang berlainan (Panadol Normal, Panadol Actifast, Panadol Muscle and Joint, Panadol Kids, Panadol Soluble, Bayer Aspirin, Alka-Seltzer, Disprin dan G-Asp Tablet) dengan menggunakan Kromatografi Cecair Prestasi Tinggi fasa-berbalik. Penceraian disiapkan dalam masa < 6 min. Keputusan analisis menunjukkan bahawa sebatian aktif yang terkandung dalam Bayer Aspirin, Alka-Seltzer, Disprin dan G-Asp Tablet ialah asid salicilik asetil dengan kepekatan sebanyak 181.31 mg/g, 15.84 mg/g, 63.87 mg/g, dan 10.11 mg/g dalam rumusan masing-masing. Bayer Aspirin mempunyai kekuatan analgesik yang paling tinggi kerana mempunyai kandungan asid salicilik asetil yang paling tinggi.

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SYMBOL, ABBREVIATION AND UNIT LIST

mg	Milligram
%	Percentage
g	Gram
mg kg^{-1}	Milligram per kilogram
nm	Nanometer
μm	Micrometer
mL min^{-1}	Milliliter per minute
mm	Millimeter
mL	Milliliter
min	Minutes
mg L^{-1}	Milligram per liter
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Among the various analytical techniques, high-performance liquid chromatography (HPLC) constitutes the most popular chromatographic method for separating mixtures of analgesic drugs (Di Pietra *et al.*, 1996), and in the determination of drugs in biological fluids and dosage forms (Salgado & Lopes, 2006), due to its high separation efficiency, sensitivity and reproducibility (Qi *et al.*, 2002); in particular, HPLC methods have been used for analysis of acetylsalicylic acid, propyphenazone, paracetamol, caffeine (Di Pietra *et al.*, 1996; Tzanavaras & Themelis, 2006) and chlorpheniramine (Di Pietra *et al.*, 1996).

Although in some respects there are advantages of micellar electrokinetic chromatography (MEKC) over liquid chromatography (LC) including the ability to perform simultaneous analysis of drug combination with different polarity and hydrophobicity, capillary electrophoresis (CE) is often considered less sensitive than LC (Boonkerd *et al.*, 1995) owing to the minute amount of sample introduced into the capillary and the small volume of the detector cell.

Pain is a disabling accompaniment of many medical conditions, and pain control is one of the most important therapeutic priorities. Pain is a subjective experience, hard to define exactly. Typically it is a direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause (e.g. trigeminal neuralgia), or persist long after the precipitating injury has healed. It can also occur as a consequence of brain or nerve injury. The two components of which may be involved in pathological pain states: (i) the peripheral nociceptive afferent neuron, which is activated by noxious stimuli; (ii) the central mechanisms by which the afferent input generates a pain sensation.

Analgesics are medicine or drugs that reduce, relieve or eliminate pain. There are basically two kinds of analgesics: non-narcotics and narcotics. Non-narcotic analgesics work by affecting the prostaglandin system, which is the system within the body responsible for producing pain. On the other hand, narcotic analgesics can be divided into two types: the opiates and the opioids, which is the derivative of opiates. Opiates are the alkaloids found in opium. Narcotic analgesics are prescription only medicines that are very potent, being chemically related to morphine. Effective analgesia is capable of modifying many of the pathophysiological responses to injury, thereby assisting recovery (Kehlet, 1999; Kehlet & Dahl, 2003).

Analgesics include paracetamol (acetaminophen), non-steroidal anti-inflammatory drugs (NSAIDs) such as salicylates, narcotic drugs such as morphin, synthetic drugs with narcotic properties such as tramadol and various others. Analgesic drugs act in various ways on the peripheral and central nervous system.

Non-narcotic analgesics act peripherally by blocking pain signals going to the brain or by interfering with the brain's interpretation of the signals, without producing anesthesia or loss of consciousness, unlike opioids that depress the central nervous system (CNS) and inhibit the brain's ability to feel pain. Analgesics will not treat the cause of the pain but they will provide temporary relief from pain symptoms. NSAIDs relieve pain and also reduce inflammation, unlike acetaminophen that provides pain relief but does not reduce inflammation. Analgesics are widely used not only as pain relievers but also in several diseases (arthritis, rheumatism). Their determination in biological fluids (overdose monitoring) and in pharmaceutical dosage forms (quality control) remains of great interest (Di Pietra *et al.*, 1996).

Acetaminophen, also known as paracetamol, is the most commonly used over-the-counter non-narcotic analgesic. Structurally, acetaminophen is similar to aspirin. They are both recognized by the same enzyme, cyclo-oxygenase (COX). COX serves as a pain activator, amplifying the degree of pain experienced in order to let the body know that there is a problem. Acetaminophen, however, only inhibits prostaglandin biosynthesis in the central nervous system with little or no effect on peripheral tissues. Acetaminophen is a popular pain-reliever because it is both effective for mild to moderate pain relief and relatively inexpensive. Acetaminophen works as a weak prostaglandin inhibitor. It is also often used in combination with other narcotic drugs in some of the stronger analgesics. Acetaminophen can also be found in combination with other active ingredient in many cold, sinus, and cough medications.

Aspirin is a member of the NSAIDs. It is originally derived from the organic compound salicylic acid that accumulates in the willow tree. However, the main

problem with salicylic acid, apart from its unpleasant taste, was that it was harsh on the stomach. Hence, this problem is addressed by synthesizing acetylsalicylic acid, which is a chemical salt that neutralized the salicylic acid. Aspirin has many therapeutic qualities. It is an analgesic that can reduce pain as well as an anti-inflammatory that can alleviate swelling. It is used in the treatment of a wide variety of ailments including headache, acute migraine, fever (pyrexia), muscular pain and many others.

Caffeine, 1, 3, 7-trimethylxanthine, is the major alkaloid ingredient in about 60 herbs, including *Thea sinesis* (tea leaves), *Coffee arabica* (coffee beans), and *Cola nitida* (kola nuts) (Abourashed & Mossa, 2004). Caffeine may be the most popular drug in the world. Caffeine is also a central nervous system stimulant. Caffeine is a common ingredient in many painkillers and anti-migraine pharmaceuticals (Abourashed & Mossa, 2004). The pharmacological effect of caffeine can be achieved when it is consumed in the form of herbal extract or pure ingredient added to various food products. In massive doses caffeine is lethal. Some studies have shown that caffeine causes physical dependence. Typical withdrawal symptoms associated with caffeine are headache, fatigue and muscle pain. These symptoms can occur within 24 hours after the last dose of caffeine (Tzanavaras & Themelis, 2006).

1.2   Objectives of Study

The objectives of the study are:

- To determine the active compound (phenacetin, acetylsalicylic acid and caffeine) in analgesic formulation using HPLC.
- To quantify the active compounds.

1.3   Scope of Study

The study involved preparation of sample solutions, tablets used were commercial tablet formulations. The samples were nine commercial tablet formulations (Panadol Tablets, Panadol Soluble, Panadol Muscle and Joint, Panadol Kids, Panadol Actifast, Bayer Aspirin, Disprin, Alka-Seltzer and G-Asp Tablets) containing certain amounts of active compounds. The samples prepared were then analyzed using the HPLC technique.

CHAPTER 2

LITERATURE REVIEW

2.1 Narcotic and non-narcotic analgesics

2.1.1 Introduction

A medication is any drug taken to cure or reduce the symptoms of an illness or ongoing medical condition. Medication can be usually classified in various ways, e.g. by its chemical properties, mode of administration, or biological system affected. There are many types of medication; this includes those for the cardiovascular system, central nervous system, respiratory system, allergic disorders and also for pain and consciousness, which are the analgesic drugs.

2.1.2 Narcotic analgesics

The narcotic analgesics, also termed opioids, are all derived from opium and are the primary classes of analgesic. Snyder (1973) find direct evidence that opiods are recognized by specific receptors, though the existence of specific antagonists had earlier suggested that such receptors must exist. Now confirmed by receptor cloning, is that three types of opioid receptor (Dhawan *et al.*, 1996), termed μ, δ and κ (all of

them typical G-protein-coupled receptors), mediate the main pharmacological effects of opiates, as summarized in Table 2.1. Recent studies on the characteristics of transgenic mouse strains lacking each of the three main subtypes show that the major pharmacological effects of morphine, including analgesia, are mediated by the μ-receptor (Rang *et al.*, 2003).

Table 2.1 Functional effects associated with the main types of opioids receptor. (Rang *et al.*, 2003)

	μ	δ	κ
Analgesia			
Supraspinal	+++	-	-
Spinal	++	++	+
Peripheral	++	-	++
Respiratory depression	+++	++	-
Pupil constriction	++	-	+
Reduced GI motility	++	++	+
Euphoria	+++	-	-
Dysphoria	-	-	+++
Sedation	++	-	++
Physical dependence	+++	-	+

Opioids vary not only in their receptor specificity but also in their efficacy at the different types of receptor. Thus some agents act as agonists on one type of receptor and as antagonists or partial agonists at another, producing a very complicated pharmacological picture (Rang *et al.*, 2003). Three main categories may be distinguished: Pure agonists, partial agonists and mixed agonist-antagonists, and antagonists.

Pure agonists include most of the typical morphine-like drugs. They all have high affinity for μ-receptors and generally lower affinity for δ- and κ-sites. Some

drugs of this type, notably codeine, methadone and dextropropoxyphene, are sometimes referred to as weak agonists, since their maximal effects, both analgesic and unwanted, are less than those of morphine, and they do not cause dependence. Whether they are truly partial agonists is not established.

Partial agonists and mixed agonist-antagonists are typified by nalorphine and pentazocine and combine a degree of agonists and antagonists activity on different receptors. Nalorphine, for example, is an agonist when tested on guinea-pig ileum, but it also inhibits competitively the effect of morphine on this tissue. In vivo, it shows a similar mixture of agonist and antagonist actions. Pentazocine and cyclazocine, by comparison, are antagonists at μ-receptors, but partial agonists on δ- and κ-receptors. Most of the drugs in this group tend to cause dysphoria, rather than euphoria, an effect mediated by the κ-receptor or the non-opioid σ-receptor (Rang *et al.*, 2003). On the other hand, antagonists are drugs that produce very little effect when given on their own but block the effects of opioids. The most important examples are naloxone and naltrexone.

2.1.3 Effects of narcotic analgesics

Although treatment of mild to moderate pain can typically be accomplished with non-narcotic analgesics such as acetaminophen or aspirin, treatment of severe pain often requires use of an opioid analgesic such as morphine (Burger, 2003). The narcotic analgesics vary in potency, but all are effective in treatment of visceral pain when used in adequate doses. They reduce or block the activation of pain neurons in the gray matter of the spinal cord, and at the receptor sites in the brainstem and thalamus.

They are indispensable drugs in the management of cancer pain, and also for the treatment of chronic pain.

The effects of opium, from which morphine is isolated, have been known for thousands of years, but it is only within the twentieth century, and really within the last 25 years, that we have begun to understand the effects of opioid analgesics at a molecular level. Beckett and Casy (1954) proposed that opiate affects were receptor mediated, but it was not until the early 1970s that the stereo specific binding of opiates to specific receptors was demonstrated in mammalian brain tissue.

The most important effects of morphine are on the central nervous system (CNS) and the gastrointestinal tract, though numerous effects of lesser significance on many other have been described. Opium is effective in most kinds of acute and chronic pain, though opioids in general are less useful in neuropathic pain syndromes than in pain associated with tissue injury, inflammation or tumour growth. It also reduces the affective component of pain. This reflects its supraspinal action, possibly at the level of the limb system, which is probably involved in the euphoria-producing effect.

Opium causes a powerful sense of contentment and well-being. This is an important component of its analgesic effect, since the agitation and anxiety associated with a painful illness or injury are thereby reduced (Rang et al., 2003). The euphoria produced by opium depends considerably on the circumstances. In the patients who are distressed, it is pronounced, but in patients who become accustomed to chronic

pain, opium causes analgesia with little or no euphoria. Some patients report restlessness rather than euphoria under these circumstances.

Euphoria appears to be mediated through μ-receptors and to be balanced by the dysphoria associated with κ-receptors activation. Thus different opioid drugs vary greatly in the amount of euphoria that they produce. It does not occur with codeine or with pentazocine to any marked extent, and nalorphine, in doses sufficient to cause analgesia, produces dysphoria.

2.1.4 Codeine

While morphine is the most powerful medical analgesic substance, there are many other naturally occurring alkaloids derived from opium. The best known of these is codeine. Codeine is a heterocyclic compound which exerts its effects by acting on the central nervous system (brain and spinal cord). It is an alkaloid found in opium in concentrations ranging from 0.3 to 3.0 percent. While codeine can be extracted from opium, most codeine is synthesized fro morphine through the process of O-methylation.

Codeine's action is weaker than that of morphine, and therefore less likely to lead to drug addition. However, common to all opiates is the attribute that if they are taken for weeks or months the recipient will need larger doses to obtain the same analgesic and sedative effects. If given in doses of 60 mg with acetaminophen, codeine results in additional pain relief but may also increase the incidence of side effects (Moore *et al.*, 1998).

2.2   Non-narcotic Analgesics

2.2.1   Non-Steroidal Anti-inflammatory Drugs (NSAIDs)

The NSAIDs are medications other than corticosteroids that relieve pain, swelling, stiffness, and inflammation, but they do not cure the diseases or injuries responsible for these problems. NSAIDs inhibit prostaglandin synthetase, there by reducing the process of inflammation. NSAIDs are an important component in balanced analgesia in the management of acute and chronic pain. As a group, they are all effective analgesics.

NSAIDs have a direct action on spinal nociceptive processing with a relative order of potency that correlates with their capacity to inhibit cyclooxygenase (COX) activity. The two isoforms of cyclooxygenase, COX-1 and COX-2, are genetically distinct, with COX-1 located on chromosome 7 and COX-2 on chromosome 1 (Wallace & Staats, 2006). COX-1 is considered constitutive or part of the basic constitutional homeostasis, while COX-2 is inducible; that is, it responds to specific insult. Various NSAIDs inhibit the isoforms differently.

Although NSAIDs act primarily through their affects on peripheral prostaglandin synthetase, additional central mechanisms for their action have also been demonstrated. Clinically, NSAIDs have an important role as adjuvant to other analgesics and have an opioid-sparing effect in the range of 20-35% (Wallace & Staats, 2006). When given in combination with opioids after surgery, NSAIDs result

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