CERTIFICATION OF APPROVAL

Mercury Compound Detection in Cosmetic Products

By

Rawani binti Mohamed Razali

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Approved by,

Azry Borhan
Lecturer
Chemical Engineering Programme
Universiti Teknologi PETRONAS
31750 Tronoh, Perak, MALAYSIA.
Tel: 605 3721258 (DL) Fax: 605 3721111
E-mail: azryb@petronas.com.my

(Azry Borhan)

UNIVERSITI TEKNOLOGI PETRONAS
TRONOH, PERAK
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CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowlegdements, and the original work contained herein have not been undertaken or done by unspecified sources of persons.

RAWANI BINTI MOHAMED RAZALI

ABSTRACT

Mercury-containing cosmetics have been represented for many years as skin bleaching agents in skin whitening products, or as preparations to remove or prevent freckles and/or brown spots. The trade in skin whitening creams is booming in Britain, and the creams are enormously popular in many African countries, particularly post-apartheid South Africa, as well as amongst African American women. However, women are poorly informed about the effects of using skin whitening products. The objective of this research study is to investigate the presence of mercury compounds in whitening facial cream and facial cleanser by performing experimental analysis. There is also a need to research for the allowable limit of mercury in the cosmetics and finally to research for the effects of mercury-containing whitening cream to consumers. The analysis of mercury compound was carried out to six skin whitening products from various brands. The presence of mercury compounds was determined by using the method of vaporization and trapping with Atomic Fluorescence Spectrometry (AFS). From the experimental results, the actual mercury concentration in each sample products was calculated and was presented in parts per million (ppm) levels. Product C contains the highest mercury concentration, which is 329.88 ppm, followed by Product D with 267.53 ppm, then Product F with 136.39 ppm, Product B with 65.86 ppm, Product A as the second lowest with 26.01 ppm, and finally the lowest mercury-containing product is Product E. Since there should be no any trace amount of mercury in cosmetic products except not more than 65 ppm of mercury used as preservative in eye area cosmetics, the amount of mercury in all sample products obtained, are all exceeding the allowable limit. However, the results obtained cannot be approved since the method used to dissolve or to extract the sample products was not based on any approved standard such ASTM Standard Method. Furthermore, there was no Certified Reference Material was used to compare the experimental results with the theoretical values. As to conclude, there is mercury compound exist in skin whitening products but the concentration differs amongst products. The experimental analysis of can be improved by employing a standard method of cosmetic analysis, and student must be more precautious during the preparation of samples.

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CHAPTER 1
INTRODUCTION

1.1 BACKGROUND OF STUDY

Over the last few years, cosmetic market has been one of the most innovative and challenging sectors of the personal care industry, generating significant amounts of research and development prodded by the increased growth in demand. Increasing consumer concerns about health and a feeling of wellness have triggered explosive growth of 10 to 15 percent per year for cosmetic intensifying competition to produce unique active ingredients and higher performance cosmetic products [(1)].

Cosmetic products are classified into 13 categories by Food and Drugs Administration (FDA) [(1)], for example facial cleanser, facial moisturizer, make up remover, deodorant and shampoo. For this research project, the cosmetic product to study is the skin whitening products, specifically whitening facial cream and facial cleanser.

The trade in skin whitening creams is booming in Britain, and the creams are enormously popular in many African countries, particularly post-apartheid South Africa, as well as amongst African American women [(2)]. In fact, while Westerners spend cash topping up their tans to appear attractive, many Asians are slathering on creams to reduce skin colouring as they embrace a different concept of beauty that for them says white is right .

Studies by market research company, Synovate Inc. says sales of skin whitening products in Asia are soaring as the region's beauty-conscious try to lose the pigmentation they consider unattractive [(2)].

Nearly half of Hong Kong women surveyed by the company last year bought such treatments, up from 38 percent in 2002. Whitening creams were also bought by more than a one third of females in Indonesia, Malaysia and Taiwan [4]. According to Japanese cosmetics giant Shiseido, sales of skin-whitening products in Asia grew by 20 percent between 1997 and 2003 and accounted for 23 percent of the company's total sales in the region [4]. In Thailand, the whitening lotion segment accounts for more than 60 percent of the country's annual 100 million US dollars facial skincare market [4]. Another study found that 69 percent of Indonesian males and 65 percent of females preferred fair complexions among the opposite sex as did more than 74 percent of males and over half of females surveyed in Malaysia [3].

According Bernice Tse, Product Manager of L'Oreal Paris skincare products in Hong Kong, said that Asians define beauty definitely different from that of the Western countries [4]. Nowadays they are not only asking for no freckles, they neither want the yellow color on their face. The 'white' concept is core. The extent of the popularity of skin-whitening products could be seen by the wide range of products available in market that began booming in 2000. They all claim to be able to eliminate skin pigment, bleach the skin or lighten skin tone and dark spots. Among them are OLAY White Radiance Purifying Cloths, L'OREAL White Perfect Triple Whitening Body Moisturizer, PONDS Double White Eye Stick, NIVEA Whitening Toner and NEUTROGENA Fine Fairness Essence [4]. Those products are easily to be found in Malaysian market too. In late 2002, whitening products also found their way into deodorant roll-ons in Indonesia.

If we look at narrower market scope, among female students in University Technology Petronas itself, it can be found that among 100 persons that had been interviewed, 61 persons are applying whitening cream on their faces however with products from various local and international manufacturers. Most products claimed that they give encouraging instant result. But do these products safe? Thus we have to look at the ingredient or the whitening agent in the whitening cream.

Mercury-containing cosmetic preparations have been represented for many years as skin bleaching agents or as preparations to remove or prevent freckles and/or brown spots (so-called age spots) [5]. Preparations intended for such use are regarded as drugs as well as cosmetics. In addition to such use as skin-bleaching agents, mercury compounds have also been widely used as preservatives in cosmetics such as hand and body creams and lotions; hair shampoos, hair sets and rinses, hair straighteners, hair coloring, and other preparations; bath oils, bubble bath, and other bath preparations; makeup, antiperspirants and deodorants; and eye-area cosmetics [5].

Mercury, also called quicksilver and hydrargyrum, is a chemical element in the periodic table that has the symbol Hg and atomic number 80. A heavy, silvery, transition metal, mercury is one of only three elements that are liquid at room temperature (the others are bromine and gallium). Mercury is used in thermometers, barometers and other scientific apparatuses. Mercury is mostly obtained by reduction from the mineral cinnabar [42].

The toxicity of mercury compounds is extensively documented in scientific literature. It is well known that mercury compounds are readily absorbed through the unbroken skin as well as through the lungs by inhalation and by intestinal absorption after ingestion. Mercury is absorbed from topical application and is accumulated in the body, giving rise to numerous adverse effects. Mercury is a potent allergen and sensitizer, and skin irritation is common after topical application[5]. Cosmetic containing mercury compounds are often applied with regularity and frequency for prolonged periods. Such chronic use of mercury-containing skin-bleaching cream has resulted in the accumulation of mercury in the body and the occurrence of severe reactions [5].

All this while, the concentration of mercury can be accurately determined in air, water, soil, and biological samples (blood, urine, tissue, hair, breast milk, and breath) by a variety of analytical methods [6].Whereas the detection of mercury concentration in cosmetics is very limited. Some analytical methods also require the pre-digestion of the sample prior to the reduction to elemental mercury.

Most methods use Atomic Absorption Spectrometry (AAS), Atomic Fluorescence Spectrometry (AFS), or Neutron Activation Analysis (NAA), although Mass Spectrometry (MS), Spectrophotometry, and Anodic Stripping Voltammetry (ASV) have also been employed [7]. The most commonly used method is Cold Vapour (CV) AAS (ATSDR, 1999). Through CVAAS, mercury concentrations below the microgram per liter or microgram per kilogram level can be reliably (more than 76% recovery) measured through either direct reduction of the sample or reduction subsequent to pre-digestion [7]. Electrothermal AAS has been demonstrated to be highly sensitive and to produce excellent accuracy (ATSDR, 1999). Sub-microgram per liter or microgram per kilogram range sensitivity and excellent accuracy have also been demonstrated with gas chromatography (GC) or microwave-induced plasma atomic emission detection (Bulska et al., 1992). Recovery of more than 90% and high precision have also been obtained with AFS when the samples were predigested in a closed container in a microwave oven (Vermeir et al., 1991a, b). ASV and isotope-dilution spark source MS, which also require pre-digestion of the sample, have also produced high precision and accuracy (recoveries more than 90%). Inductively Coupled Plasma–Atomic Emission Spectroscopy (ICP-AES) and ICP-MS can also be used to accurately (more than 90% recovery) determine total mercury in blood and urine with sub-microgram per liter sensitivity, but with less precision [7].

1.2 PROBLEM STATEMENT

Each day, women reach for facial cleanser, purifying toner, moisturizer, whitening cream, and UV protection cream before the dusting powder. Cosmetic products they had used are actually exposing themselves to about 200 different chemicals daily. In the majority of cases, women are poorly informed about the chemical substances they are dealing with. People are given the impression that lighter skin is a passport to better relationships and success, whereas, women in the west hasten to the tanning salons believing the more tanned you are, the healthier you look. Therefore, the quest for altering skin color is it lighter or darker, is ultimately detrimental to health. Consequently, despite the confused emotional, mental and psychological attitudes of women, or rather because of it, skin care especially whitening creams particularly targeted to the Middle East, Asia and Africa will continue to dominate cosmetic production, accounting for 60% the industry by 2007 [8].

Dermatologists say these products bleach the skin by breaking down the skin's melanin, and destroying the skin's protective layer in the process. Eventually the skin will start to burn, itch or blister and become extremely sensitive to sunlight ultimately turning darker than before [8]. Other side effects of bleaching include severe acne, stretch marks, thinner skin which bruises easily, loss of protection against harmful sun rays, blisters containing blood may form, dark red, brown or white spots and even leading to a loss of vision [8]. Moreover, heavy metals like mercury have been found in some skin lightening soaps, which can result in disorders of the kidney [9]. Prolonged use can damage the nerves or even lead to skin cancer and liver toxicity, proving to be fatal [8].

Until the 70s, mercury compounds even more toxic than hydroquinone were included in soaps and skin-lightening creams, but they were banned because of the damage they caused to both the skin and internal organs. However trading standards officers have found that some illicit skin lightening products still contain mercury, while others boast of this metal but actually contain none - a con that can protect skin [2].

Doctor Michael Chan of Hong Kong's Prince of Wales Hospital said that some unscrupulous vendors are selling products containing dangerously high level of mercury [4].

From a study conducted by two Arabic dermatologist (Iman Al-Saleh, Inaam Al-Doush) on "Mercury Content in Skin Lightening Creams and Potential Health Hazards to the Health of Saudi Women", found that 26 skin whitening creams from other countries that contained mercury, and even exceeded up to more than 1000 ppm, as shown in Table 1.1 below.

Table 1.1: Mercury-Containing Skin Whitening Creams

Product name	Mercury (ppm)	Country of origin	Color
Kelly	0.43	Indonesia	Shiny yellow
Orrefor (Extra pearl Cream)	912.50	Unknown	Dark shiny yellow
Pally (Nourishing Cream)	2.19	Thailand	Light yellow
Bivong	928.50	Unknown	Dark shiny yellow
Butae (Pearl Cream)	517.50	Unknown	Shiny yellow
Ginseng (Extra Pearl Cream)	467.35	Unknown	Yellow
ARCHE (Formula A.A. Melasma Cream)	0.74	Thailand	Pale yellow
Last fade cream (The secret of seaweed)	0.60	Indonesia	Shiny white
Ginseng (Extra pearl cream)	594.50	Unknown	Shiny yellow
Emoon (Pearl Grease)	0.45	Unknown	Pale yellow
U.B. Formula 99 A.A. (Melasma Cream)	0.43	Thailand	Pale yellow
Silvana cream	2.15	Lebanon	Creamy white
Ly-NA (Nourish face cream)	1.49	Taiwan	Shiny orange
El-Arais cream	0.23	Syria	Pale white
Hawaa cream	0.00	Syria	Shiny white
Hawaa cream	0.00	Syria	Shiny pink
Ly-NA (Medicated pearl paste)	0.00	Taiwan	Shiny orange
Paris cream	0.00	United Arab Emirate	Shiny white
ALFA (Extra pearl cream)	1319.00	Unknown	Shiny yellow
Yin Fong (Extra pearl cream)	388.90	Thailand	Shiny yellow
Rose cream	0.00	Lebanon	Shiny beige
Yong Chin (Nourishing cream)	0.00	Thailand	Off-white
Beanne (Pearl cream)	0.09	Thailand	Dark shiny yellow
Diana	5650.00	Lebanon	Dark shiny beige
Civic (Nourishing cream)	1964.50	Thailand	Pale yellow
Ideal cream	1.18	Lebanon	Shiny beige
Melanax	1.73	Pakistan	Shiny white
Bashaer	0.73	Syria	White

Cing-Cing	0.13	Unknown	Pale yellow
Cing-Cing (Roon Petch)	1.18	Unknown	Pale yellow
Daifu (Herbal formula/pearl cream)	271.15	Thailand	Shiny off-white
Drula (Bleaching wax)	0.00	Germany	Shiny white
Tibet Snow	0.37	Pakistan	Shiny white
Palmer's Skin Success (Fade cream)	0.00	England	Shiny white
Skinicles (Fade cream)	0.00	England	Shiny white
Fade-Out	0.00	England	Shiny white
Cream Minerva	1281.50	England	Shiny beige

In Hong Kong in 2002,one woman was admitted to hospital and 13 others referred to specialists after they used one of two whitening creams that had mercury levels between 9,000 and 65,000 times recommended levels [(4)].

A study of 38 skin-whitening creams by Hong Kong's Chinese University chemical pathology professor Christopher Lam Wai-kei in 2000, showed eight made by global cosmetic makers exceeded the US Food and Drug Administration safety limits for mercury [(4)].

High doses of mercury are associated with sight or hearing loss and hand tremors as well as personality changes, anxiety, insomnia, memory loss, progressing to cerebral palsy and potentially fatal kidney failure [(7)].

The detection of mercury compounds in the whitening creams needed to obtain more concern by many parties, and the consumers especially women out there are well-informed about the findings rather than letting them endangered their skin and body by using those whitening creams, whether with or without significant mercury concentration.

1.3 OBJECTIVES OF THE PROJECT

This research project serves for several prominent objectives, which are:

1. To investigate the presence of mercury compounds in whitening facial cream and facial cleanser by performing experimental analysis.
2. To investigate the allowable limit of mercury in the cosmetics.
3. To investigate the effects of mercury-containing whitening cream to consumers.

1.4 SCOPE OF WORK

The research study is focusing on the determination of an appropriate method to detect the presence of mercury compound in the cosmetic cream then the research shall be accomplished by performing an experimental analysis. From the experiment, mercury concentration in the samples shall be determined. The result is then compared with the allowable limit of mercury concentration in cosmetic, affirmed by the government. The research is lengthened by finding the health hazards or effects that mercury-containing creams can bring about.

1.5 METHODOLOGY

In order to carry out the scope work stated above, the methodologies are:

1.5.1 Early Stage

Gathering of information regarding cosmetics, hazardous compounds contained in cosmetics and health issues due to usage of cosmetics were done mostly by surfing to the Internet. The frequent used search engines were Yahoo! and Googles. There were also some articles from News Strait Times were referred to. The research was continued on finding the standard experimental cosmetic analysis or any analysis that had been studied but it was very limited.

1.5.2 Middle Stage

Since no finding on mercury detection in cosmetic product from the Internet and related books, initiative was taken to get the exact information on mercury detection needed from the right persons. The best place to go is the research center and the best research center to go is the PETRONAS Research and Scientific Services (PRSS). Discussion with a mercury-expert chemist, Dr Azman bin Shafawi was carried out. Experimental analysis to detect mercury content in the whitening creams and facial cleansers was performed as well. Before that, the selection of the product to be analyzed was based on the one that was familiar to Malaysian consumers with affordable price. The sample product was dissolved with several solvents in order to find the solvent that could dissolve the sample cream well.

1.5.3 Final Stage

Mercury concentration in each product in parts per million (ppm) levels was determined by calculations and completion of final dissertation. Research however was continued throughout the report completion.

CHAPTER 2
LITERATURE REVIEW

2.1 COSMETIC

2.1.1 Cosmetic Categories

The manufacturers of cosmetics group cosmetics into several categories [7]:

1. skin cosmetics: creams, soaps, lotions, powders, and colors accessories
2. hair cosmetics: shampoos, lotions, oils, waving agents, fixatives, bleaches, dyes, and dye removers
3. nail cosmetics: lotions, polishes, colors, and accessories
4. hygiene and psyche cosmetics: deodorants, antiperspirants, hair removers, oral preparations, sun tan products, perfumes, and lipsticks.

As for skin cosmetics, the types of products falls under this category are such as facial cleanser, facial moisturizer/treatment, acne treatment/medication, make-up and make-up remover, and anti-aging treatment.

2.1.2 History of Cosmetics [10]

Cosmetics is general term applied to all preparations used externally to condition and beautify the body, by cleaning, coloring, softening, or protecting the skin, hair, nails, lips, or eyes. Perfumery is usually excluded from the field of cosmetics, although perfumes are commonly manufactured in coordination with cosmetics.

The use of cosmetics is worldwide and dates from the remotest antiquity. Although it is generally believed that cosmetics as they are now known originated in the Far East, the study of non-industrial cultures indicates the use of cosmetics in every part of the world. The war paint of Native Americans, the tattooing and *scarification* (making of superficial incisions of the skin) practiced by many peoples (the Maori of New Zealand and numerous African cultures), and the use of woad (a plant dye used by ancient Britons to paint their bodies blue) are all forms of cosmetic used for psychological intimidation of the enemy as well as adornment.

The earliest known cosmetics come from the first Dynasty of Egypt (about 3100-2907 BC). Tombs of this era have yielded unguent jars, and from remains of later periods it is evident that the unguents were scented. Such preparations, as well as perfumed oils, were extensively used by both men and women to keep the skin supple and unwrinkled in the dry heat of Egypt. Egyptian women also developed the art of decorating the eyes by applying dark green color to the lower lid and by blackening the lashes and the upper lid with *kohl,* a preparation made from antimony or soot.

By the middle of the first century AD, cosmetics were widely used by the Romans, who employed kohl for darkening eyelashes and eyelids, chalk for whitening the complexion, rouge and *depilatories* (hair-removing preparations), and pumice for cleaning the teeth. In the Middle Ages the Crusaders found cosmetics widely used in the Middle East, and it was they who spread the use of cosmetics throughout Europe.

The almost universal use of cosmetics in modern times has grown with the scientific study of the ingredients employed. This research was begun by the French in the 19th century, and led to the development of more and better cosmetics at low cost.

A large variety of cosmetics is generally available today. Cold cream is an emulsion of various oils and waxes and water; it is employed to cleanse and soften the skin. Various purpose-made moisturizers and cleansers are also available. Face powder and dusting powder, based on *talcum* (powdered magnesium silicate) and zinc oxide, are used to dry and give the skin a satin-like texture. Lip color, either applied directly as a lipstick or brushed on

to the lips, is made of cocoa butter or lanolin, and is manufactured in an endless variety of shades, as are rouges, mixtures of red pigments and starch or finely powdered clay. Bath salts and other bath preparations combine water-softening agents such as sodium carbonate or borax with perfume; bath oils are also a popular skin-softening and perfuming aid. Nail polishes are lacquers or plastics available in many colors. Hair lotions and sprays are used to condition the hair, keep it in place, or make it glossy. Shampoos are based on soap or synthetic detergents.

Hair-coloring dyes, tints, and rinses, available in many shades and colors, are widely used cosmetic products. *Henna* is a vegetable dye, used for centuries to impart a red tint to the hair. Weak solutions of hydrogen peroxide are often employed as hair bleaches. For coloring the eyebrows and eyelashes, mascara is generally used. This is a compound of gum and black, brown, green, or blue pigment. Sulphides of calcium and barium, which remove hair from the skin, are generally the active agents in cosmetic depilatories. Bronzes are creams that impart a color to the skin similar to that of suntan.

Cosmetics and perfumery are by no means confined to use by women, as might be assumed. Grooming aids frequently used by men include powders, colognes, and lotions, particularly alcohol-based aftershave lotions; hair tonics, often with an alcohol or quinine base; and deodorants.

2.1.3 Hazardous Chemicals in Cosmetics

The Hazardous Chemical Information Act defines a hazardous chemical as any element, chemical compound or mixture of elements and/or compounds which poses a physical hazard or a health hazard. A chemical is a health hazard if there is statistically significant evidence based on at least one study conducted in accordance with established scientific principles that acute or chronic health effects may occur are: carcinogens, irritants, reproductive toxins, corrosives, sensitizers, radioactive material, neurotoxins (nerve), biohazards, hepatotoxins (liver), nephrotoxins (kidney), agents that damage the lungs, skin, eyes, or mucus membranes.

Government scientists recently identified a group of toxic chemicals known as phthalates in urine of adults, with highest levels in premenopausal women, resulting from inhalation and skin exposure to volatile parent ingredients used extensively as solvents and plasticizers in personal care and cosmetic (PCC) products.

2.1.4 Effects of Hazardous Substances in Cosmetics [11, 12, 13]

Concerns on cancer risks from PCC products are emphasized by: lifelong use of multiple products by the majority of the U.S. population; the ready skin absorption of carcinogenic ingredients, further increased by detergents, especially when left on the skin for prolonged periods. Recent issues in cosmetic ingredients that are carcinogenic are as follows:

1. Cosmetic grade talc is carcinogenic in experimental animals. Also, frequent genital dusting with talc, routinely practiced by some 17% of women, increases risks of ovarian cancer.
2. A group of widely used preservatives, such as quaternium-15 and bronopol, widely used in baby products, though not carcinogenic themselves, break down to release formaldehyde, a potent irritant and carcinogen.
3. Lanolin, widely used on babies' skin, is commonly contaminated with DDT (dichlorodiphenyltrichloroethane) and other carcinogenic pesticides.
4. Commonly used detergents and foaming agents, such as polysorbates and PEG (polyetheleneglycol), are usually contaminated with the volatile carcinogen dioxane, although this could be easily removed by vacuum stripping during manufacture.
5. DEA (diethanolamides), another widely used chemical detergent, has been known since 1975 to combine with nitrite preservatives or contaminants in cosmetic products to form a highly carcinogenic nitrosamine. Furthermore, recent government studies showed that DEA itself is also carcinogenic following application to mouse skin.
6. Propylene Glycol (PG), Polyethylene Glycol (PEG), Butylene Glycol (BG) and Ethylene Glycol (EG) are all petroleum derivatives that act as solvents, surfactants, and wetting agents. They can easily penetrate the skin, and can weaken protein and cellular structure. PG is used in Anti-Freeze, Brake and Hydraulic Fluid , De-Icer, Paints and Coatings, Floor Wax , Laundry Detergents , Pet Food, Tobacco,

Cosmetics, Toothpastes, Shampoos, Deodorants, Lotions, Processed Foods and many more personal care items.

7. FDA issued a consumer warning that commercial "skin peel" products, advertised to remove wrinkles, blemishes, blotches and acne scars, could destroy the upper layers of the skin, causing severe burns, swelling, and pain. The active substances meant are the active skin peel ingredients — alpha and beta hydroxy acids (AHAs and BHAs).
8. The use of mercury compounds as cosmetic ingredients is limited to use as preservatives in eye area cosmetics at concentrations not exceeding 65 ppm (0.0065%) of mercury calculated as the metal (about 100 ppm or 0.01% of phenylmercuric acetate or nitrate) and provided no other effective and safe preservative is available for use. Mercury compounds are readily absorbed through the skin on topical application and have the tendency to accumulate in the body. They may cause allergic reactions, skin irritation or neurotoxic manifestations.

2.2 SKIN WHITENING SYSTEM

Skin Whitening System helps lighten the skin by inhibiting the production of melanin. It is the accumulation of melanin in the skin that causes dark skin color and age spots. As the skin naturally renews itself every 28 days, old pigmented cells are sloughed off and cells with less melanin are brought to the surface giving the skin a lighter, more even toned complexion.

On a molecular level, melanin is produced in the body from the conversion (in several steps) of the amino acid tyrosine. This conversion requires the enzyme known as tyrosinase.

Range of skin whitening system varies upon the manufacturers' selection of products that can be people's choice. Generally, skin whitening system can be consisted of facial cleanser, purifying toner, whitening cream or lotion, and finally the UV protector cream. Some whitening set may include exfoliating cream or peeling cream.

2.2.1 Common Ingredients in Skin Whitening Cream [14, 15]

There are two main ways how the ingredients in skin whitening creams whiten the skins:

- By absorbing the UV rays, thus preventing the sun rays from darkening the skin
- By inhibiting the production of melanin

Here are some ingredients that act as whitening agent that are may be found in the cream:

- ***Kojic Acid.*** It is used to lighten the skin in Japan. It is a tyrosinase inhibitor. That would explain how it reduces the production of skin pigment, melanin.
- ***Licorice Extract.*** This absorbs the UVA and UVB rays. It also is a depigmenting agent and inhibits the production of melanin.
- ***Aloe Vera.*** Aloe Vera appears to absorb UV light. That would account for its mild skin lightening abilities.
- ***Octyl-p-methoxycinnamate.*** This is a sunscreen. It absorbs UV light very well, preventing the UV rays from darkening your skin.
- ***Octyl salicylate.*** This is a salt of the salicylic acid that is found in wintergreen leaves. It is a sunscreen, found in sun tan lotions.
- ***Oxybenzone.*** This is a sunscreen chemical that absorbs UVA rays.
- ***Alpha Hydroxy Acid (AHA).*** Is used to exfoliate the skin. AHAs remove dead skin cells that dull the skin's appearance. AHAs are mild acids derived from natural substances. Citric acid from fruit, lactic acid from milk and glycolic acid from sugar cane are AHAs.
    - ***Citric Acid.*** Citric acid is obtained from fruit. Lemon, for example contains citric acid. That is why lemon is well known as a skin lightener and is often used at home in a toner to freshen oily skin. It can be very irritating to sensitive skin though.
    - ***Lactic Acid.*** Lactic Acid is commonly added to cosmetic products labelled as AHA products. Lactic acid is alpha hydroxy propionic acid. Lactic acid is also used in skin treatments. It is obtained from milk. A word of caution though. lactic acid is known to irritate sensitive skin.
    - ***Glycolic Acid.*** Glycolic acid is alpha hydroxy acetic acid. Glycolic acid is obtained from sugar cane. This is less likely to irritate skin. It is used by many dermatologists in light peels. It is also found in many cosmetic products.

- ***Hydroquinone.*** Which is now banned and can lead to irreversible hyper pigmentation – extreme darkening of the skin. It is a leading ingredient found in most modern skin-lightening products, and first tried in the 1930s by some African Americans who found they could use it to fade discoloration. The chemical works by hindering the creation of melanin; causing dark colors to fade as older cells are replaced by bleached ones [16]. Hydroquinone, thought by some scientists to be a possible carcinogen, the substance is also used in the development of photos. Manufacturers of Hydroquinone creams insist they are safe, but in 1995 the U.S., called for a ban on them [16].

2.2.2 Dangers of Skin Whitening Cream

In the 1950s and 60s, skin sanding was a very common phenomenon in the world. Soft sandpaper was used to gently scrape the face, to rub out 'blotchy' skin or freckles [8]. The skin was then treated with disinfectant and medicated cream. When the skin healed, it would have (for a short time) a fresh and delicate appearance. However, there was no scientific foundation for this method, in fact, it increased health risks by lowering the skin's resistance to infection. Today's skin lightening methods allegedly operate by reconstructing skin cells and removing toxins from the body, thus purifying the skin and giving it a fresh-faced appearance.

Humans have obstructing walls between the layers of the skin, which aid in keeping out viruses or bacteria. Common skin care products cannot safely *penetrate* the skin; thus have a temporary, superficial and psychologically uplifting effect. There is no product, not even a detergent, on the market that can turn Dark skin into Caucasian skin without irreversible damage to the skin itself [8].

2.3 MERCURY DETECTION

2.3.1 History of Mercury

Mercury was known to the ancient Chinese and Hindus and was found in Egyptian tombs that date from 1500s BC [42]. By 500 BC it was used to make amalgams with other metals.

The ancient Greeks used mercury in ointments and the Romans used it in cosmetics [42]. Alchemists thought it to be the stuff from which all matter was formed and they also thought that when it hardened it turned into gold.

In the 18th and 19th centuries, mercury nitrate was used to remove fur from the animal skins from which felt hats were made. This caused many cases of brain damage among hatters, or milliners.

It was named by alchemists after the Roman god Mercury [42]. Its symbol Hg comes from hydrargyrum, a Latinised form of the Greek word hydrargyros, which was a compound word whose Greek roots meant 'water' and 'silver' [42].

2.3.2 Method of Detection

In general the expression 'trace' can be considered as a concentration below 100 $\mu g\ g^{-1}$. Element-specific detection techniques such as atomic absorption spectrometry (AAS), atomic emission spectrometry (AES), and atomic fluorescence spectrometry (AFS) are widely used for the determination of mercury.

Atomic absorption spectrometry is the term used when radiation usually in the range 180 to 800 nm, is absorbed by the atom under measurement. The term emission spectrometry is applied to the measurement of light emitted from a flame or plasma by chemical species after the absorption of energy as heat or as chemical energy (i.e. chemiluminescence). If only the emission from atoms is observed, the term atomic emission spectrometry is preferred. The re-emission of radiation from an atom having previously absorbed light is termed atomic fluorescence.

The determination of mercury by cold vapour atomic absorption detection was first published in 1968 [17]. Since then, the determination of mercury at $\mu g\ ml^{-1}$ levels or less has received considerable interest. The importance of sampling and sample storage as well as methods of analysis has also been acknowledged. The use of AAS has emphasized the sensitivity and

ease of application [18]. Atomic absorption was used as a replacement technique to an earlier calorimetry technique [19]. Non-flame atomic absorption and fluorescence spectrometry, with sample introduction which includes pyrolysis, furnace techniques, combustion, and reduction-aeration (cold vapour), were simple and sensitive, but experienced difficulties in giving accurate determinations in natural samples [19]. The determinations were mainly in environmental materials, but some coverage of the analysis of food and biological materials was also undertaken.

Detectors based on conventional AAS or a dedicated instrument designed for the purpose offer far superior performance. Since this method is based on the absorption of a specific wavelength of light by atomic mercury, spectral interference is very unlikely. This instrument is very reliable, gives reproducible results and has a good detection limit. One disadvantage is that it is a very expensive outlay for use as a dedicated detector compared with other techniques.

The technique of AFS is more sensitive than AAS. These instrument offer high precision and accuracy, are easy to operate and are less expensive than AAS, but they do required argon gas for operation.

Table below summarizes some common methods or techniques used to determine mercury species, which can be seen that they also differ in terms of detection limit.

Table 2.1: Various Methods of Total Mercury Determination

Method	Determination	Detection Limit (ng)
Cold Vapour Atomic Absorption (CVAAS)	Hg generated after dissolution of sample	0.01
Cold Vapour Atomic Fluorescence (CVAFS)	Hg generated after dissolution of sample	0.0001
Inductively Couple Plasma Mass Spectrometry (ICP-MS)	All species in solution	0.1

Nippon Instrument Corp. SP-3D Mercury Analyser (NIC)	Double amalgamation of Hg generated by controlled combustion followed by CVAAS	0.1
Sir Galahad Mercury Analyser	Amalgamation of Hg followed by CVAFS	0.0001
Jerome Gold Film Meter	Amalgamation of Hg and recording change of conductivity of film	0.5
Wickbold/Lingener Combustion followed by CVAAS	Hg generated after combustion and dissolution of sample	0.01
Spark Source Mass Spectrometry	Hg generated by controlled combustion and followed by MS	0.1

CHAPTER 3

DETERMINATION OF TOTAL MERCURY COMPOUND IN SKIN WHITENING PRODUCTS BY VAPORISATION AND TRAPPING WITH ATOMIC FLUORESCENCE DETECTION

3.1 INTRODUCTION

The technique is based on vaporisation of the sample and trapping of elemental mercury at an elevated temperature prior to its determination by atomic fluorescence spectrometry (AFS) using the Total Mercury Analyzer [20] (a modified equipment by Dr Azman Shafawi, published in Analyst 1999). These instrument offers high precision and accuracy, easy to operate, and less expensive than Atomic Absorption Spectrometry (AAS), but they do require argon gas as carrier gas in the operation. The skin whitening products used for the analysis were of four whitening facial creams and two facial cleansers from various brands. The AFS technique offers lower detection limit thus is suitable in the determination of mercury species, which exist relatively in small concentration in the cosmetics compared to natural gas.

3.2 THEORY

It is known that mercury species are efficiently adsorbed onto gold, gold-coated materials and the platinum group metals (amalgamation) [21, 22]. As in the natural gas, the determination of total mercury can be carried out accurately and to very low limit of detection (0.0001 ng) by collecting the species onto special gold-coated sand traps at room temperature. The trapped or adsorbed mercury is released when heated to a high temperature (~ 900 °C), and

swept through into the atomic fluorescence detector by argon gas for measurement [23, 24]. The performance of different adsorbents in collecting mercury species, such as activated charcoal, silver and gold-coated *sand* has been studied by Dumarey *et al.* [21]. Elemental mercury, inorganic mercury ($HgCl_2$), organomercury halides and the di-alkyl mercury compounds generated in a stream of air (vapour form) were 100% collected when using gold coated sand trap.

Prior to the determination of mercury by an element specific detector such as AFS or AAS, the collector or trap is required to be heated to a temperature high enough to ensure the decomposition of the compounds and the release of mercury in its elemental form. The thermal desorption behaviour of several mercury species released from a gold trap, as elemental mercury is controlled by the decomposition process [25]. To obtain full desorption and decomposition, the collector or trap must be heated to at least 500°C [21, 25-27]. Mercury species will not be decomposed or released from the trap if a temperature of 250°C [28] is not reached. At 345°C, 60% of elemental mercury is released if oxygen is used as carrier but for argon or nitrogen, a temperature of 250°C is sufficient.

Atomic fluorescence spectrometry is an analytical technique used to determine the concentration of many elements in samples [29]. This technique was studied as early as 1902 by Wood, and by Nichols and Howes [30] who looked at fluorescence in flames. In 1964, Winefordner and Vickers investigated the possibility of using atomic fluorescence as a practical analytical technique [31]. They used metal vapor discharge tubes as sources and were able to obtain sensitivities of better than 1 g ml^{-1} for mercury, zinc, cadmium and thallium in an acetylene-oxygen flame. The development of a continuous source added another dozen elements to the list of possibilities [32]. Since then, several studies have been carried out on the development of AFS both in the area of analytical capability or analytical application for real samples and in the refining of instrumentation including conventional source excited AFS [33-36].

Thompson et al. pioneered the determination of mercury using AFS in the 1970s [37]. The instrument described was a dispersive system based on a modified FAAS and offered a

detection limit of 0.02 $g\ l^{-1}$. Increased light gathering power of non-dispersive system of Non-dispersive AFS is often offset by background scatter from the flame atom cell. Godden and Stockwell [38], who developed a filter fluorimeter that took advantage on the fact that mercury in atomic form is a vapor at room temperature, and therefore does not require a flame to generate atomic species, overcame this. As mercury vapor is monoatomic, a flame for mercury atomization is unnecessary if the mercury in the sample can be converted to the elemental form. Four main methods have been used to bring mercury into the vapor phase; reduction-aeration, direct heating, electrolytic amalgamation and direct amalgamation [39]. Reduction-aeration, by means of a reducing agent and sweep gas is by far the most popular. Use of this method improves detection limits by two or three orders of magnitude, compared with those based on simple flame atomization methods.

At present, CVAFS and CVAAS are the most widely used techniques for the determination of mercury. The advantages of AFS are that it is relatively cheap and simple to operate. In the absence of particulate/aerosol carry over from the CV technique and flames, there is no interference from source scattering, which contributes to its low limit of detection (LOD) and high sensitivity. However, the ability to perform multi-element determinations is not yet available. Automated mercury analyzers, based on AFS, are however commercially available and several systems can be obtained allowing solid, liquid and gas samples to be analyzed.

3.3 EXPERIMENTAL EQUIPMENT AND INSTRUMENTATION

For determination of mercury compound in sample of cosmetic products, the equipment that can be used is Total Mercury Analyzer (Analyst 1999), using the AFS technique. The equipment is integrated with a Pyrolyzer or vaporization chamber, a Trapping System Oven, a Mercury Detection System, and Touchstone Data Gathering System.

Pyrolyser that operates at high temperature converts the mercury species to elemental mercury (Hg°). The elemental mercury is detected by AFS detector. The fluorescence beam able to detect mercury species at picogram level.

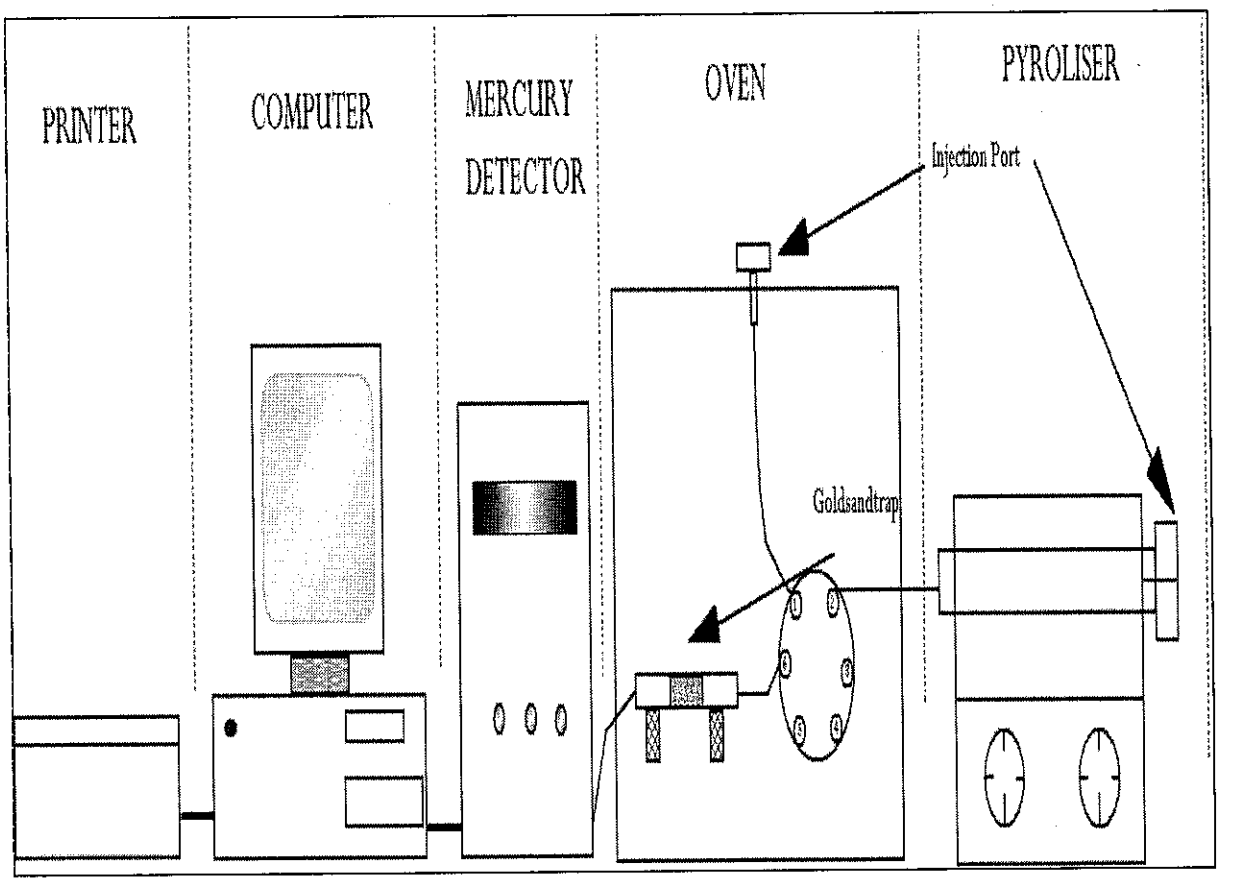


Figure 3.1: Schematic Diagram of Total Mercury Analyzer of Dr Azman Shafawi

3.3.1 Pyrolyzer (Vaporization chamber)

The chamber consists of a 250 ml three necked round bottom flask which is maintained at 400°C ± 10°C using an electrothermal heating mantle. The separate necks are connected to the heated trap line and an argon purge gas line while the third neck is fitted with a double septum for sample introduction by an injection technique. The top part of the chamber is insulated with aluminium foil to reduce heat loss. The tubing from the chamber to the gold-coated silica trap is maintained at 200°C by heating tape and a variac supply. The latter prevents the condensation of the vaporized sample before it reached the heated trap.

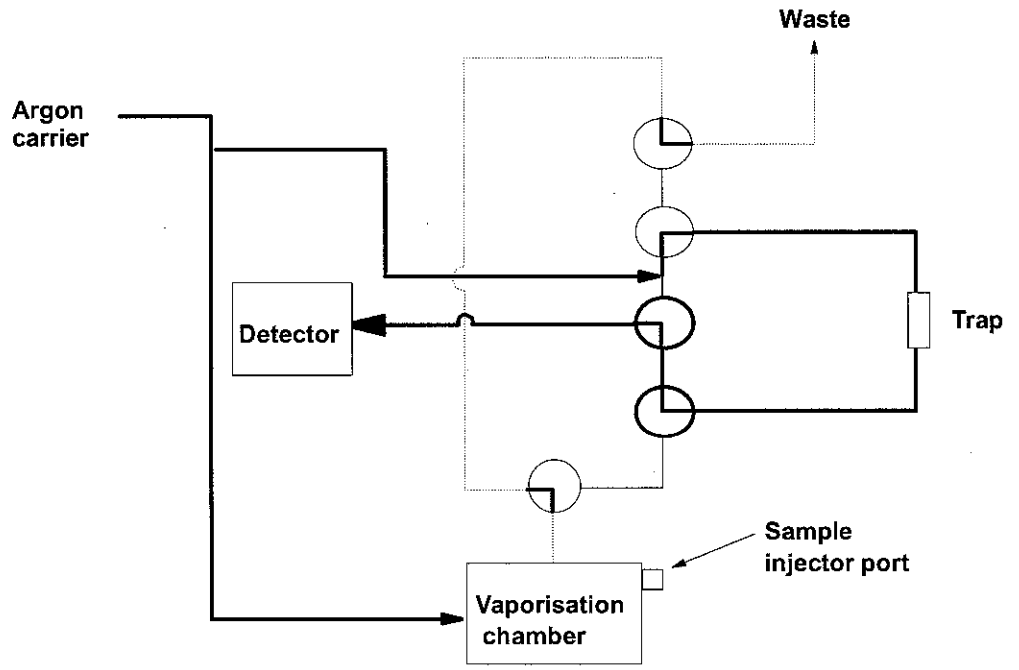
3.3.2 Adsorption trap module

The new adsorption trap module consists of a gold-coated silica (Amasil, 30 mg) bounded by Quartz wool within a silica tube and surrounded by a nichrome heating wire (to release mercury at 900°C). This tube is retained within a specially designed cooling chamber (flushed using air to return the inner silica tube to 200°C from 900°C). The trap was positioned within a small oven (Kenwood, Hants, UK) maintained at 200°C ±5°C.

3.3.2 Valve switching sequences

Control switching of the purging, cooling and carrier gas lines is performed by a computer driven 'Galahad' system (P.S. Analytical, Kent, UK). A schematic diagram of the switching arrangement for the sampling mode (pre-concentration) and for the measurement mode (detection) is shown in Figure 3.2.

(A) Sampling Mode



(B) Measurement Mode

Figure 3.2: Valve switching sequence between (A) sampling mode (pre-concentration) and (B) measurement mode

3.3.4 Filter

To improve baseline stability and prevent trace organic material entering the detector system, a filter is inserted into the gas line prior to the detector. This filter, which comprises of two ashless, No.1 filter papers in a 2 cm diameter demountable holder (Whatman Int. Ltd., Maidstone, UK), did not affect the performance of the calibration or any subsequent analyses. It will be changed for every fifty run or earlier if shown to be necessary.

3.3.5 Process Description of the Equipment

An accurately measured volume of sample is injected using a gas-tight syringe (Dynatech Precision, Baton Rouge, Louisiana, USA) into the three necked vaporisation chamber and held at a temperature of 400°C. Normally some 5 to 10 minutes is required to vaporise the sample completely; a parameter which had been studied before. The sample vapour generated is continuously swept by argon gas, at between 300 and 400ml per minute, through to the gold-coated silica trap that is maintained at 200 °C within the small oven.

The sample matrix is consequently carried in its vapour phase away from the trap and is directed to a waste collector. The mercury species which is first adsorbed on the gold coated silica trap, is subsequently released (as elemental mercury) by heating to a temperature of 900°C and swept through to an atomic fluorescence detector (Merlin, P.S. Analytical, Orpington, Kent, UK). The results in form of volatogram will be displayed by the Touchstone Data Gathering System. The Touchstone Software Package is used in capturing the fluorescence signal and calculating the concentration of mercury based on the calibration curve being used. Example of volatogram is shown in Figure 3.3 below.

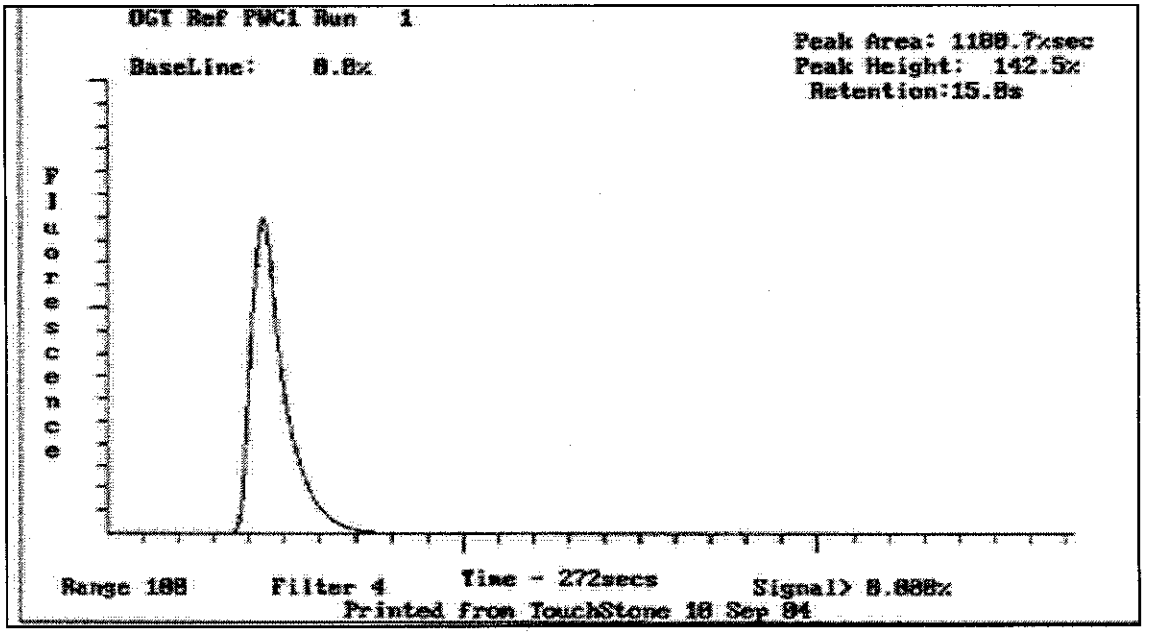


Figure 3.3: Example of Volatogram

3.3.6 Calibration procedure.

Calibrations is based against elemental mercury for all species. The calibration relies upon the knowledge that at a fixed temperature, the saturated vapour pressure of mercury is known and a fixed volume of vapour will contain a known quantity of mercury. This volume is injected and adsorbed onto the gold sand trap and then re-vaporised into the detector where the peak response is measured [40]. Once the values of temperature and volume are known, the absolute quantity of mercury adsorbed onto the trap can be calculated.

3.3.7 Apparatus

1. 50 ml volumetric flask
2. Specimen bottle
3. Filter paper
4. Gas-tight syringe

3.3.8 Reagent

Ethanol (Analytical Grade)

3.3.9 Experimental Parameters

Table below summarizes the optimum condition of the parameters of the integrated Total Mercury Analyzer.

Table 3.1: Experimental Parameters

Parameters	Values
Vaporisation Chamber Temperature (^{o} C)	400
Vaporisation Time (min.)	5 - 10
Argon Carrier for Vaporisation (ml min $^{-1}$)	300 - 400
Argon Flow Rate for the Detector (ml min $^{-1}$)	500
Detector Sheath Gas Flow Rate (ml min $^{-1}$)	250
Gold Trap Flushing Time (sec.)	30
Gold Trap Vaporisation Time (sec.)	15
Gold Trap Vaporisation Temperature (^{o} C)	900
Gold Trap Cooling Period (min)	2
Cold Trapping Temperature (^{o} C)	200

3.4 EXPERIMENTAL PROCEDURE

3.4.1 Procedure: Determination of Solvent

The most important thing to do before proceeding with experiment to trace mercury in the whitening cream is to determine the appropriate solvent for the creams. Trial and error method can help in determining the solvent that can well-dissolved a portion of the cream in the specimen bottle. There were 11 types of solvents had been tested which were:

a) Aqua Regia Acid
b) Ketone
c) n-hexane
d) xylene
e) toluene
f) sodium hydroxide
g) soap solution
h) hydrogen peroxide
i) ethanol
j) dimethyl ketone
k) nafta condensate type KR2 HSRN

3.4.2 Procedure: Equipment Setting

1. Carrier gas is turned on to 30 psi and the compressed air at 40 psi by opening the valve at the gas cylinder.
2. The printer, computer, Sir Galahad Analyzer, Oven and the Pyrolyzer are switched on. The Oven is set at 190°C.
3. The TSLOAD Program is accessed. The New Calibration Curve or Amend Curve is done.
   3.1. Go to Menu/Library, press enter, 'select' press enter, choose the 'file name', press enter.
   3.2. Appropriate data in the relevance space is filled up by pressing F4.
   3.3. A new standard calibration curve is established by choosing 'Calibration' in the menu bar. Highlight 'New Curve', press enter.
   3.4. New Calibration Template will appear, Mercury Calibration Unit is chosen, press enter; peak height is chosen, press enter; number of run, Mercury Vapor temperature, volume of injection of Standard (L) are entered.
   3.5. Galahad Trap Clean is abort, press Y for yes when the first 'Setting Correct..." command appear.

3.6. Certain volume of mercury vapor for standard 1 is introduced in to the pyrolyzer injection port, with lever gear to 'Sampling' mode. And time for 5 minutes.

3.7. After 5 minutes, the lever gear is turned to 'Analysis' mode and press Y for yes to the second 'Setting Correct …' command. Analysis is going on.

3.8. Step 3.6 and 3.7 are repeated for the next standard concentration until a calibration curve is obtained.

4. After the calibration, it is ready for analyzing the sample.

3.4.3 Procedure: Preparation of Sample Solution

1. All apparatus must be cleaned up with distilled water for several times before using to prepare the samples. All preparation steps will be done in the fume hood.
2. The cosmetic cream is weighted to ±1.000 g by using the digital weighing machine and is kept in the specimen bottle with the cap tightly closed.
3. The ±1.000 g cream is dissolved with ethanol by shaking vigorously. The solution is transferred into 50 mL volumetric flask and make up with ethanol to 50 mL and is shaken again. The volumetric flask is washed with ethanol for several times before filling it up with sample solution. The volumetric flask with the sample solution is dipped into the Ultra Sonic 3210 Brandson to further dissolve the aqueous sample.
4. The sample solution is filtered with a filter paper and the filtrate is kept in the specimen bottle with the cap tightly closed.
5. Sample is ready to be analyzed.

3.4.4 Procedure: Sample Analysis by Total Mercury Analyzer

1. Go to Menu/Library, press enter, 'select' press enter, choose the 'file name', and press enter. 'Analyse' is taken and press enter. 'Single' is selected and press enter.
2. Key in for the 'Reference' and press enter.
3. Volumes of sample that will be injected is keyed into the 'Gas Sample Volume' command (L) and press enter.
4. Abort 'Galahad Cleaning' by pressing any key or enter key.

5. 'Setting Correct…' command will appear and press Y for yes and second command will appear, hold on for sample injection.
6. Liquid sample is taken (100 or 200 L – depends on the concentration of the sample) by using a syringe and is injected into the injection port at pyrolyzer. Ensure the syringe is washed with the aqueous sample for several times before using it. And timing for 5 minutes with lever gear to 'Sampling' mode.
7. After 5 minutes, lever gear is turned to 'Analysis' mode and press Y for yes to the second 'Setting Correct …' command. Analysis is going on.
8. The analysis is repeated for at least 6 times for each sample.

CHAPTER 4
RESULT & DISCUSSION

4.1 OBSERVATION

From the experiment to find the solvent for the cream, it had been observed that the skin whitening creams dissolved very well in ethanol compared to other ten solvents but sample solutions were cloudy and small particles still existed. However, as the sample solutions were dipped into the Brandson Ultrasonic, the solution dissolved completely (small particle disappeared). The cloudy solutions became very clear after the filtration was done. The whitening creams did not dissolve in sodium hydroxide, ketone, n-hexane, hydrogen peroxide, soap solution, dimetyl ketone, and condensate at all.

Table 4.1: Experimental Parameters of Each Sample

Item	Product A	Product B	Product C	Product D	Product E	Product F
Weight of Product (g)	0.9924	0.9984	1.0082	1.0062	1.0035	1.0049
Volume of Ethanol (ml)	50	50	50	50	50	50
Volume of Injection (l)	20	20	10	10	10	10

4.2 DISCUSSION

4.2.1 On Result

The raw readings obtained are shown in Appendix 3 and accordingly mean and standard deviation from readings of each product is calculated. Standard deviation is the average distance between each data in a distribution and the mean of distribution. It represents the consistency of the data reading. Lower standard deviation shows high data consistency while higher standard deviation shows least data consistency (less precise). The highest standard deviation is given by data for Product D, which is ±7.6, and the lowest standard deviation is for Product A, which is ±0.40, while the second highest is from Product C which is ±6.5 and the others are considerably low, which means considerably precise. The inconsistency readings obtained might be due to some probable errors in experimental analysis technique which will be discussed later.

From the experimental data, average mercury concentration in each products was then calculated (sample calculation is shown in Appendix 2), and the results were then converted from ng/ml to parts per million (ppm) unit as shown in Table A2 and Table A3 (Appendix 3).

The objective of this project is to investigate the presence of mercury compound in cosmetic products. Thus, by experimental analysis using the vaporization and trapping with Atomic Fluorescence Spectrometry, the concentration of mercury compound in all sample products managed to be determined. According to the bar graph as shown in Figure 4.1, Product C contains the highest mercury concentration, which is 329.88 ppm, followed by Product D with 267.53 ppm, then Product F with 136.39 ppm, Product B with 65.86 ppm, Product A as the second lowest with 26.01 ppm, and finally the lowest mercury-containing product is Product E. From the group of products being analyzed, four of them are the whitening facial creams, which are Product C, D, E and F, and the other two of them are whitening facial cleansers, which are Product A and B. Product A is simultaneously coming from the similar brand with Product F and Product B is the same brand with Product D.

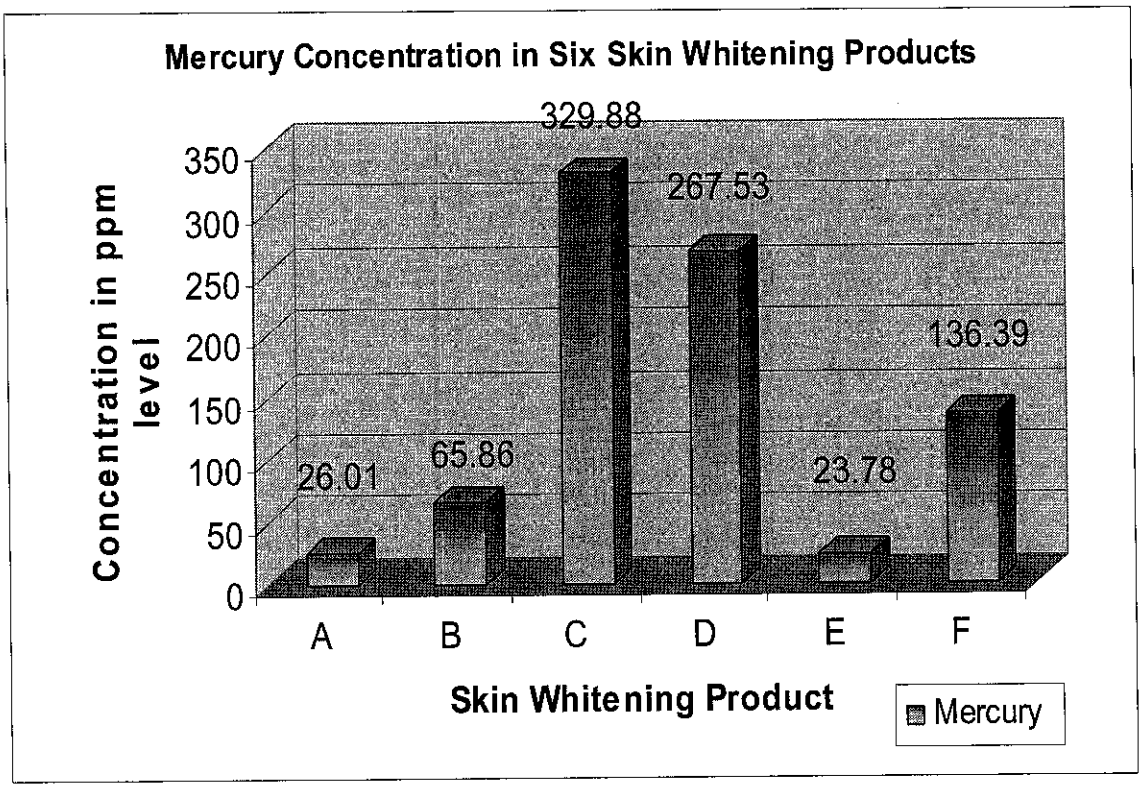


Figure 4.1: Concentration of Mercury (ppm) in Six Skin Whitening Products.

The reason of choosing product from the same brand is that to observe how the manufacturers distribute the use of mercury in their two products as both meant for skin whitening. However, as facial cleanser is milder than the facial cream, in terms of driving the effects of changing the skin color, it should contain less concentration of peeling agent in it. Besides, since facial cleanser is used more frequent than the cream, the ingredient must be less concentrated and can be seen from the texture itself. The facial cleanser is more dilute than the facial cream.

If we compare Product A and Product F, Product F has 80.9% higher mercury concentration than Product A. Same goes to Product B and D where Product D has mercury concentration of about 75.4% higher than in Product B. In other words, facial creams contain higher mercury concentration than facial cleansers. Imagine that consumers are told to apply both products sequentially, started with facial cleanser and enhance the results by applying the facial cream, therefore, they will be exposed to mercury-containing products at least twice a day. Thus, the skin too will absorb high mercury toxicity.

The AFS method is the recognized method used to determine the mercury in any matrixes. But since there is no Certified Reference Material being implemented on this cosmetic analysis, the results obtained are not an approval that the amount traced is exactly what contains in each product. For example, a cosmetic material that has a mercury concentration that had been approved by the manufacturer is analyzed under the similar condition of Total Mercury Analyzer, and the result obtained is the exactly what had been stated by the manufacturer, then the results of mercury concentration obtained from this study can be approved. Besides, the solvent used to dissolve the whitening products during the preparation of liquid sample for analysis that had been applied was not based on any ASTM (American Society for Testing Material) Standard Method.

Mercury (0) is also known as elemental or metallic mercury, and is a dense, silvery-grey liquid at room temperature. When mercury (0) is oxidized to mercury (I) and (II), it forms compounds with various electron donors. Mercury bound to a carbon atom is 'organic mercury', examples of which include methylmercury and ethylmercury. Organic mercury is always mercury (II) [4]. When not bound to carbon, mercury is 'inorganic'.

The mercury compound traced in the products is however unknown types of mercury species, whether inorganic, organic mercury or mercury salts is used as bleaching agent or as preservatives. This is because the heating at high temperature had released the mercury species into its elemental form (Hg^0) prior to the boiling point of mercury which is 356.73 °C (629.88 K) and being detected then. The most important thing is that we had found out that there is mercury species had been used as the ingredient in the skin whitening system, because it had not been stated on the packaging. No products have ever been including mercury compound in their ingredient list.

4.2.2 Allowable Limit of Mercury in Cosmetic [5]

There is no justification for the use of mercury in skin-bleaching preparations or its use as a preservative in cosmetics; with the exception of eye-area cosmetics because mercury compounds are exceptionally effective in preventing Pseudomonas contamination of

cosmetics and Pseudomonas infection of the eye can cause serious injury, including blindness.

The Food and Drug Administration withdraws the opinion expressed in trade correspondence TC-412 (issued Feb. 11, 1944) and will regard as adulterated within the meaning of section 601(a) of the Act any cosmetic containing mercury unless the cosmetic meets the conditions of paragraph (d)(2) (i) or (ii) of this section:

i. It is a cosmetic containing not more than a trace amount of mercury and such trace amount is unavoidable under conditions of good manufacturing practice and is less than 1 part per million (0.0001 percent), calculated as the metal; or

ii. It is a cosmetic intended for use only in the area of the eye, it contains not more than 65 parts per million (0.0065 percent) of mercury, calculated as the metal, as a preservative, and there is no effective and safe nonmercurial substitute preservative available for use in such cosmetic.

Besides, any product containing mercury as a skin-bleaching agent and offered for sale as skin-bleaching, beauty, or facial preparation is misbranded within the meaning of sections 502(a), 502(f)(1) and (2), and 502(j), and may be a new drug without approval in violation of section 505 of the Federal Food, Drug, and Cosmetic Act.

Due to that occasion, where there is should no any trace amount of mercury in cosmetic products except not more than 65 ppm of mercury used as preservative (mercury compound is dissimilar than the mercury compound in other types of cosmetics) in eye area cosmetics, the amount of mercury in all sample products obtained, are all exceeding the allowable limit.

4.2.3 The selection of solvent

In order to analyze the sample cosmetic creams, the sample creams must be in the form of solution. Determination of an appropriate solvent to dissolve the sample cream was

considerably challenging because it had not been included in the ASTM. From the primary literature, in cosmetic analysis, the most frequent solvents used to extract the chemical compounds in the creams are benzene and acid solution such as sulphuric acid and hydrochloric acid. In other words, the solvent for cosmetic creams can be polar or non-polar type solvents.

Generally, a good solvent meets the following criteria: a) it should be inert to the reaction condition b) it should dissolve the reactants and reagents c) it should have an appropriate boiling point d) it should be easily removed at the end of the reaction [43]. The second criterion invokes the adage "like dissolves like". Non-polar reactants will dissolve in non-polar solvents. Polar reactants will dissolve in polar solvents. There are three measures of the polarity of a solvent: a) Dipole moment b) Dielectric constant c) Miscibility with water.

Molecules with large dipole moments and high dielectric constants are considered polar. Those with low dipole moments and small dielectric constants are classified as non-polar. On an operational basis, solvents that are miscible with water are polar, while those that are not are non-polar; like "Oil and water do not mix". Examples of polar solvents are water (HOH), methanol (CH_3OH), and acetic acid (CH_3CO_2H). Examples of non-polar solvents include benzene (C_6H_6), carbon tetrachloride (CCl_4), and diethyl ether ($CH_3CH_2OCH_2CH_3$).

For this experiment, ethanol (polar solvent) dissolved all the creams very well compared to the other solvents. The whitening creams did not dissolve in sodium hydroxide, ketone, n-hexane, hydrogen peroxide, soap solution, dimetyl ketone, and condensate at all. There is a need to analyze the ethanol too as to find the mercury concentration since ethanol is a hydrocarbon. And the result of each product will need to deduct with result of ethanol.

Not all the sample products used stated the chemical substances (ingredients) contained in the cream. Only Product B, E and F displayed their ingredients. From the ingredients, propylene glycol, many types of saturated fatty acids such as stearic acid and mysristic acid, and carboxylic acid type such as lactic acid. Fatty acids and carboxylic acids both are organic acids [42], which can be dissolved by ethanol.

4.2.4 Experimental Inaccuracy

The inaccuracy of the result might have happened due to inaccuracy in executing the experiment. In dealing with mercury analysis, we can never underestimate the behavior of mercury because mercury can easily contaminate. Whenever the sample is placed in the surrounding where mercury species is within the boundary for example in the laboratory itself, the sample can be contaminated with the mercury compound if the specimen bottle did not tightly close however it had been ensure that the bottle was closed tightly during the experiment. What comes into matter is that the sample bottles had been abandoned in the lab the whole experiment time. This might make the samples were contaminated and affecting the results.

All the apparatus to be used in preparation and storage of samples, it must be cleaned thoroughly with distilled water. In the usage of the gas-tight syringe, every time to proceed with another sample, the syringe was washed with the (chemical) and rinsed with the sample solution. However, to get more accurate result, every different sample should use different syringe.

The ethanol that had been used was taken from the storage. Since the laboratory is meant to undergo all sorts mercury analysis, even the chemical taken from the storage might be contaminated. Thus to ensure a convincing result, should be using uncontaminated ethanol.

Due to unfamiliarity with the equipment, the results might be inaccurate regarding the technique of injection done by the student. Obviously it can be compared the result obtained between when the injection was done by the lab technician, who had many years experience in handling the analysis to the student. The technique of sample injection into the pyrolyzer is important since it reflects how the liquid sample will be distributed within the vaporization chamber and so along the heat transfer line. If the sample vapor is spreading inappropriately, the gold sand trap will not capture the mercury vapor efficiently.

To get a precise result, after each analysis, should run blank analysis. By running blank, any remaining sample vapor and most importantly the mercury trapped in the oven or mercury detector will be swept away by the argon gas. The blank was done until it gave 0.000 ng mercury concentration. However, it is better to repeat blank analysis for another one or two times even after the result shows 0.00 ng mercury because this can really remove any trapped from previous sample.

4.2.5 Effects of Mercury-containing Cosmetics

Dermatologists say skin whitening products bleach the skin by breaking down the skin's melanin, and destroying the skin's protective layer in the process. Eventually the skin will start to burn, itch or blister and become extremely sensitive to sunlight ultimately turning darker than before [8]. Other side effects of bleaching include severe acne, stretch marks, thinner skin which bruises easily, loss of protection against harmful sun rays, blisters containing blood may form, dark red, brown or white spots and even leading to a loss of vision [8]. It will be even more severe for which heavy metal like mercury exists in the beauty creams.

Mercury is a problem because it has the potential to be toxic in different forms and because the toxic responses are often markedly different among individuals. Although other reactions occur, the principal reaction of mercury in biological systems is with sulfhydryl (-SH) groups [41]. The predominant biological sulfhydryl compound is the amino acid cysteine. Toxicity is caused primarily by mercury binding and inactivation of cysteine residues in enzymes and structural proteins [41].

Mercury can be exposed through inhalation, ingestion, injection and cutaneous absorption. Exposure through oral ingestion requires a compound that has a degree of solubility in both aqueous and lipid environment. Exposure through inhalation requires volatility. Exposure through injection can occur due to the use of red pigments in tattoos. Cutaneous absorption is probable for mercury compounds that are lipid soluble, particularly with prolonged contact and high concentrations.

A limited amount of mercury (0) can be absorbed directly through the intact skin, but its release into the circulation is slow and of little significance [41]. Cutaneous absorption has been claimed for 'beauty creams' containing high concentrations of mercury (I) chloride [41]. While significant exposure clearly occurs with these products, it is more likely to proceed through decomposition to mercury (0) and mercury (II). Cutaneous absorption is more probable for 'skin whitening creams' containing 'ammoniated mercury' known as mercury (II) ammonium chloride (Cl-Hg-NH2) [41].

Bleaching action can take weeks or months to work and when women stop using the creams they cause hyperpigmentation - patchy or blotchy skin, which is caused by the accumulation of toxic bleaching agent [2]. If it penetrates below the outer layer into the dermis, it causes collagen fibers to thicken, leading to possibly irreversible damage to connective tissue in skin cartilage and premature aging. Once in the blood stream it can cause liver and kidney damage and lead to organ failure [2].

Use of mercury-containing beauty creams (skin-bleaching agent) has resulted in the accumulation of mercury in the body and the occurrence of severe reactions. The scientific literature shows that exposure to mercury have been associated with a variety of problems such as Alzheimer's Disease, autoimmunity, kidney dysfunction, infertility, polycystic ovary syndrome, neurotransmitter imbalances, food allergies, multiple sclerosis, thyroid problems, and an impaired immune system [7].

Mercury exposure has also been associated with other neurological problems such as tremors, insomnia, polyneuropathy, paresthesias, emotional liability, irritability, personality changes, headaches, weakness, blurred vision, dysarthria, slowed mental response and unsteady gait [7]. Generally, the target tissues are appetite and pain centres in the brain, cell membranes, kidneys, and nervous system (central and peripheral). In brain tissue, elemental mercury (Hg^0) reacts to cause neurologic impairment and is retained in brain tissue as a mercuric species [7].

Individual who exposed to mercury-containing beauty creams apparently experience signs and symptoms such as: Abnormal nervous and physical development (fetal and childhood), anemia, anorexia, anxiety, blood changes, blindness, colitis, depression, dizziness, drowsiness, emotional instability, fatigue, fever, hallucinations, headache, hearing loss, hypertension, insomnia, kidney damage or failure, loss of appetite and sense of smell, loss of muscle coordination, memory loss, nerve damage, stomatitis, tremors, vision impairment, vomiting, weakness, and weight loss [7].

CHAPTER 5
CONCLUSION & RECOMMENDATION

The increase demand of skin whitening products in Middle East, Africa and Asian countries has been triggered by the increase in feeling of wellness and fairness like the Western women. However, women are poorly informed about the consequences of prolonged application of the beauty creams onto the skin due to the hazardous chemicals used as the bleaching or peeling agent in the whitening creams.

The main objective of this project is to investigate the presence if mercury compound in the skin whitening product, besides to research for the allowable limit of mercury species in cosmetic and finally to mentions the effects of mercury-containing cosmetics to consumers. From the study, mercury species present in skin whitening products can be detected by Atomic Fluorescence Spectrometry, which is a technique of vaporization and trapping of elemental mercury at high temperature and detection under atomic fluorescence beam at 253.7 nm wavelength, using argon gas carrier gas.

From the experiment, the presence of mercury species had been successfully determined in all sample skin whitening products. However, the concentration differs from each product. Product C contains the highest mercury concentration, which is 329.88 ppm, followed by Product D with 267.53 ppm, then Product F with 136.39 ppm, Product B with 65.86 ppm, Product A as the second lowest with 26.01 ppm, and finally the lowest mercury-containing product is Product E. As to compare with the allowable limit, where there is should no any trace amount of mercury in cosmetic products except not more than 65 ppm of mercury used as preservative in eye area cosmetics, the amount of mercury in all sample products obtained, are all exceeding the allowable limit.

Indeed, the results obtained cannot be approved since there is no Certified Reference Material had been use to compare the experimental results with the theoretical values. Besides, the method used in preparation of liquid sample for analysis is not referring to ASTM Standard Method. However, it is obvious that in this study, cosmetic products do contain mercury compound.

Use of mercury-containing beauty creams (skin-bleaching agent) has resulted in the accumulation of mercury in the body and the occurrence of severe reactions. Once it is in the blood stream it can cause liver and kidney damage and lead to organ failure.

As the conclusion, the objectives of this project had been fulfilled. But there is still room for improvement especially in the experimental analysis, in order to obtain results that are more precise and accurate and the most important thing it can be approved.

To recommend on improving the experiment that had been done, the sample solution should not be keeping inside the laboratory for days. It is better to prepare the sample just when it is ready to analyze. Besides, blank analysis should be carried more than once after each sample analysis.

For further recommendation, there is a need to study the determination of mercury compound in cosmetics that can be done by using other analytical methods and apparatus. For example, from the Directive Commission of European Communities, the determination of mercury compound is carried out by using Thin Layer Chromatography (TLC). TLC uses a glass or plastic plate, where different components in the mixture move up the plate at different rates, due to differences in their portioning behavior between the mobile liquid phase and the stationary phase. By using TLC, a single test can identify several compounds of mercury species. Furthermore, the preparation of the sample is more appropriate because it will go through an extraction in the centrifuge, to give better separation of greatest part of solid from the liquid, thus taking out other unwanted components. Above of all, taking consideration of all the precaution and cleanliness during the experimental analysis on mercury is the most important job to implement.

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APPENDICES

APPENDIX 1: METHOD OF CALCULATION

Mean and Standard Deviation

The readings of every sample analysis obtained from the experiment is taken into average value or mean, by using this equation:

$$= \frac{x1 + x2 + x3 + x4 + x5}{5}$$

Standard Deviation, S is determined by using the equation below:

$$S = \sqrt{\frac{\sum(-x)^2}{n-1}}$$

Where,

$\sum(-x)$ = summation of mean minus each data

n = number of data

Determination of Mercury Concentration in Sample of Whitening Cosmetic Products

1. From the 5 readings collected for each product, the concentration of mercury in 1 ml of sample solution is determined.

$$Data1\_(ng/ml) = \frac{1\_ml}{Volume\_of\_Injection\_(\mu l)} x\,\mathrm{Re}ading1\_(ng)$$

Mercury concentration in ng/ml is obtained from all Reading 1 to Reading 5 and average value is then calculated.

2. From the average value obtained, to get the actual mercury (Hg) concentration in the sample solution, we need to deduct with the average value of mercury concentration in ethanol.

Actual Hg in Sample (ng/1 ml) = Average Hg in Sample – Average Hg in Ethanol

3. The raw product is dissolved in 50 ml of ethanol, thus determine the concentration of mercury in 50 ml sample solution.

Actual Hg in Sample (ng/50ml) = Actual Hg in Sample x 50 ml

4. In 50 ml sample solution, the weight of raw product is ±1.000 g. Thus, mercury concentration in 50 ml sample solution is equal to ±1.000 g of product.

Actual Hg in Product (ng/g) = Actual Hg in Sample (ng/50ml)

5. Mercury concentration in 1000 g (1 kg) of product is determined because in ppm level, it is mg/kg.

Hg in 1 kg Product (ng/kg) = Actual Hg in Product (ng/g) x (1000 g/Weight of Sample)

Hg in Product (ppm) = Hg in 1 kg Product x 10^6

APPENDIX 2: SAMPLE OF CALCULATION

Mean and Standard Deviation

Take Product A as example.

No	Item	Calculation
1.	Mean,	$= (x_1 + x_2 + x_3 + x_4 + x_5)/5$ $= (11.296 + 12.995 + 12.7 + 14.05 + 13.07)/5$ $= 12.8222$
2.	$(- x_1)^2$	$= (12.8222 - 11.296)^2$ $= 2.32928644$
3.	$(- x_2)^2$	$= (12.8222 - 12.995)^2$ $= 0.02985984$
4.	$(- x_3)^2$	$= (12.8222 - 12.7)^2$ $= 0.01493284$
5.	$(- x_4)^2$	$= (12.8222 - 14.05)^2$ $= 1.50749284$
6.	$(- x_5)^2$	$= (12.8222 - 13.07)^2$ $= 0.06140484$
7.	$[\sum(- x)^2]/(n-1)$	$= (2.32928644 + 0.02985984 + 0.01493284 +$ $1.50749284 + 0.06140484)/(5-1)$ $= 3.9429768/4$ $= 0.9857442$
8.	Standard Deviation, S	$= \sqrt{[\sum(- x)^2]/(n-1)}$ $= 0.992846514$

Mercury in Product in ppm Level

Take Product A as example.

No	Item	Calculation
1.	Data 1	= 1 ml/Volume of Injection x Reading 1 = 1 ml/0.02 ml x 11.296 = 564.8 ng/ml
2.	Data 2	=1 ml/0.02 ml x 12.995 = 649.75 ng/ml
3.	Data 3	= 1 ml/0.02 ml x 12.7 = 635 ng/ml
4.	Data 4	= 1 ml/0.02 ml x 14.05 = 702.4 ng/ml
5.	Data 5	= 1 ml/0.02 ml x 13.07 = 653.5 ng/ml
6.	Average	= (Data 1 + Data 2 + Data 3 + Data 4 + Data 5)/5 = (564.8 + 649.75 + 635 + 702.4 + 653.5)/5 = 641.11 ng/ml
7.	Actual Hg in 1 ml Sample	= Average Hg in Sample – Average Hg in Ethanol = 641.1 – 124.83 = 516.28 ng/ml
8.	Actual Hg in 50ml Sample	= Actual Hg in 1 ml Sample x 50 ml = 516.28 x 50 ml = 25814 ng/50 ml
9.	Hg in 1 kg Product (ng/kg)	= Actual Hg in Product (ng/g) x (1000 g/Weight Sample = 25814 ng/g x (1000 g/0.9924 g) = 26.01 x 10^6 ng/kg
10.	**Hg in Product (ppm)**	= Hg in 1 kg Product x 10^6 **= 26.01 ppm**

APPENDIX 3: RESULT OF CALCULATION

Table A3a: Mean and Standard Deviation of Experimental Data of Each Analyzed Products

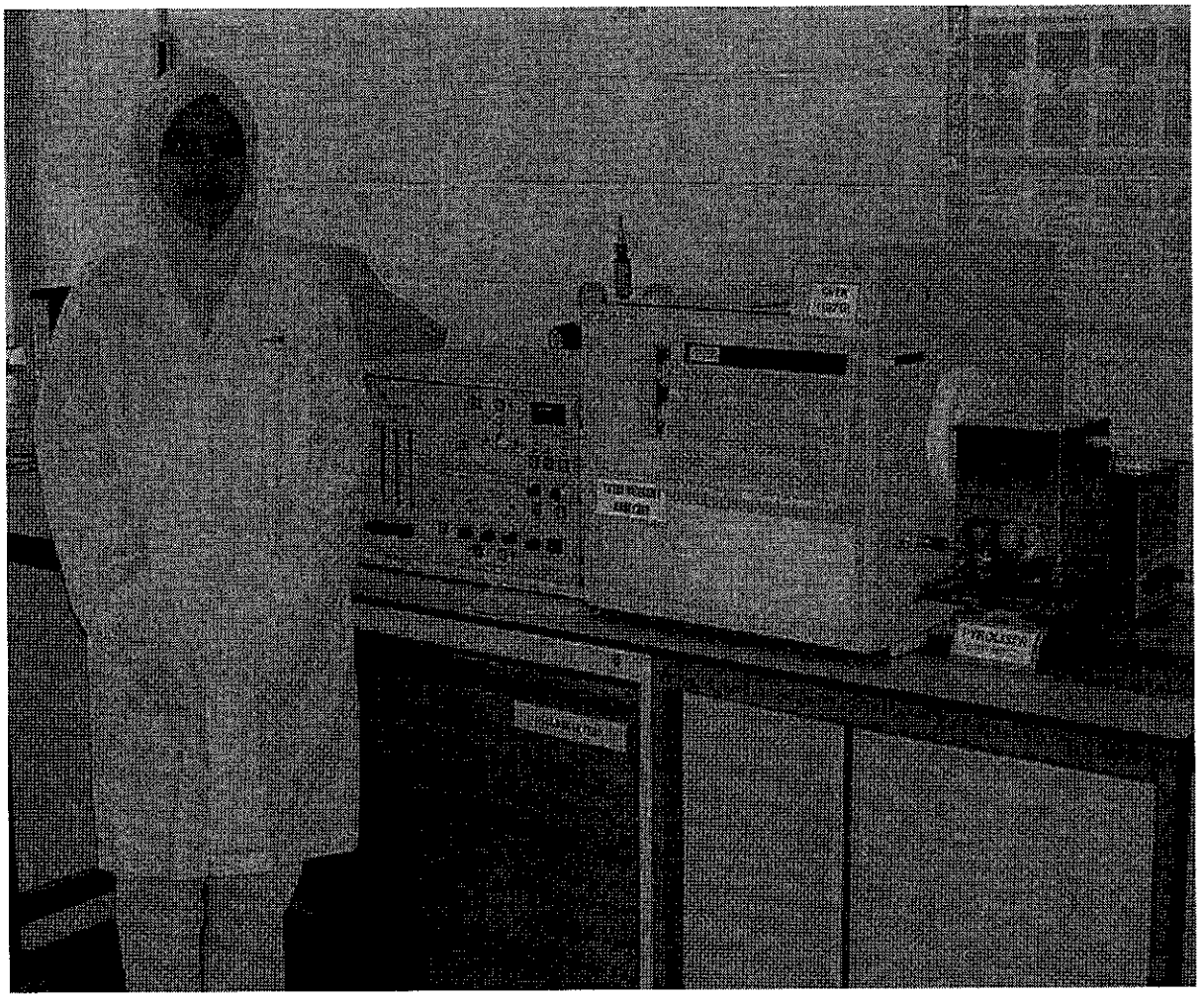
Product	Reading 1	Reading 2	Reading 3	Reading 4	Reading 5	Mean & ± s.d
Ethanol	3.09	2.08	2.17	2.61	2.53	2.50 ± 0.4
Product A	11.30	13.00	12.70	14.05	13.07	12.82 ±0.99
Product B	29.85	22.16	29.61	32.5	29.88	28.80 ± 3.9
Product C	64.09	75.86	59.65	66.77	72.46	67.77 ± 6.5
Product D	62.66	46.05	60.17	65.94	60.61	55.09 ±7.6
Product E	6.63	6.48	6.71	3.32	6.96	6.02 ± 1.5
Product F	32.32	28.20	26.80	26.93	29.05	28.66 ± 2.2

Table A3b: Average Amount of Mercury in Each Product in ng/ml

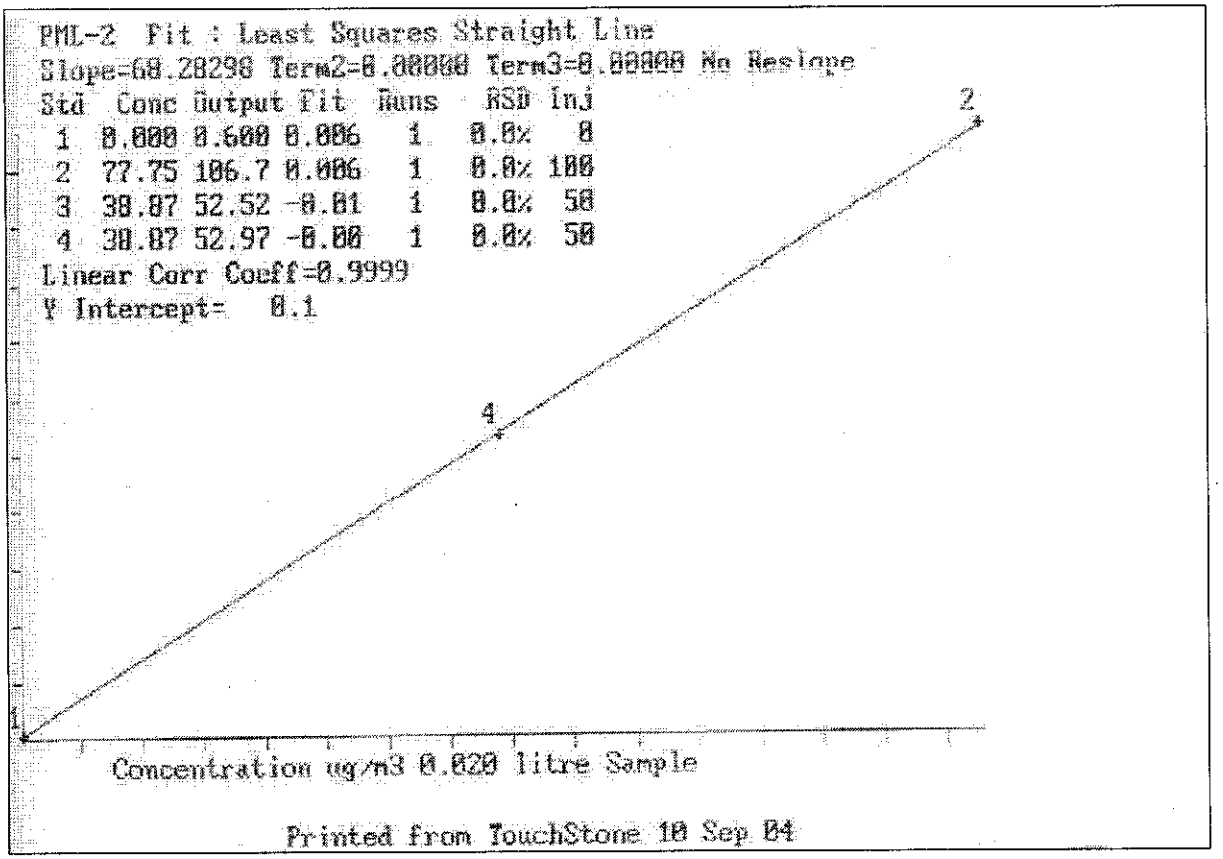
Item	Ethanol	Product A	Product B	Product C	Product D	Product E	Product F
Data 1 (x_1)	154.65	564.80	1492.50	6409.00	4266.00	663.20	3232.00
Data 2 (x_2)	104.00	649.75	1108.00	7586.00	4605.00	648.40	2820.30
Data 3 (x_3)	108.55	635.00	1480.50	5965.00	6017.00	670.90	2680.00
Data 4 (x_4)	130.50	702.50	1625.00	6677.00	6594.00	331.70	2693.00
Data 5 (x_5)	126.45	653.50	1494.00	7246.00	6061.00	696.00	2904.50
Average (ng/ml)	124.83	641.11	1440.00	6776.60	5508.60	602.04	2865.96

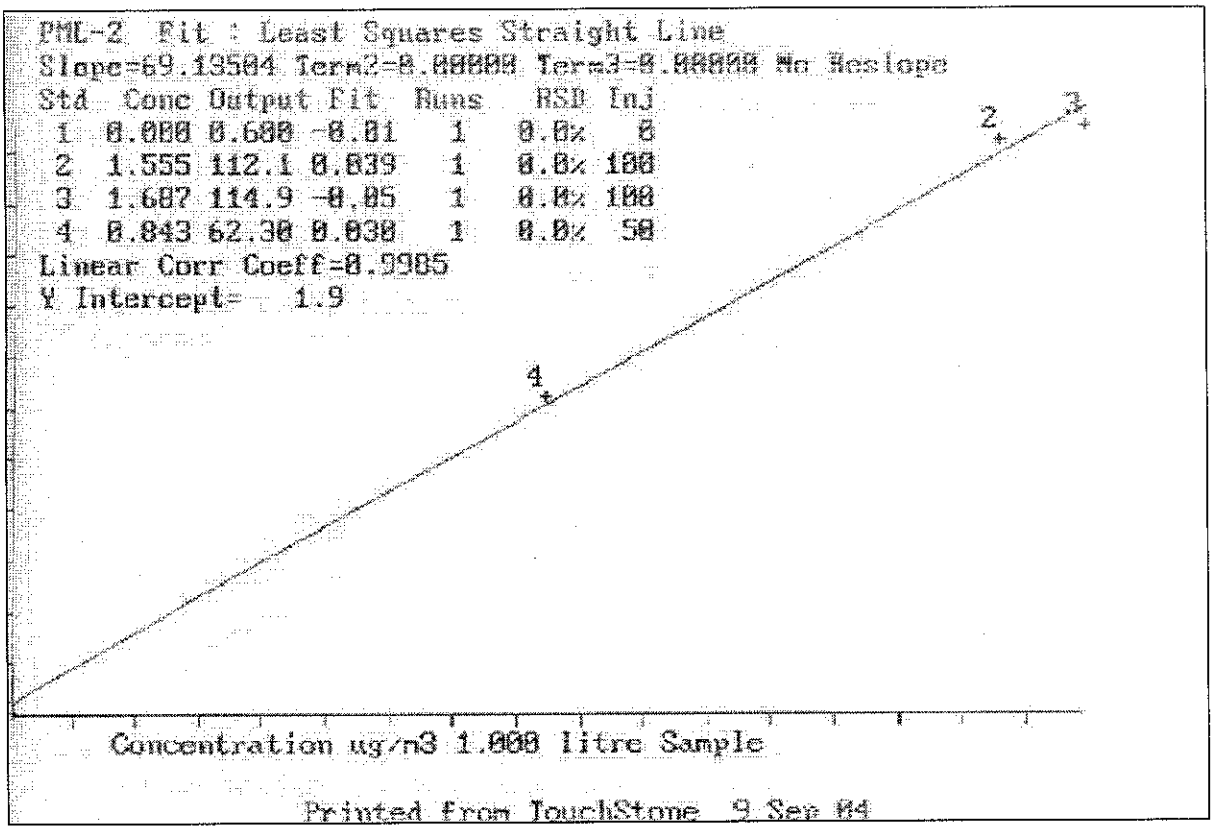
Table A3c: Concentration of Mercury (Hg) in Each Product in Different Units						
Item	**Product A**	**Product B**	**Product C**	**Product D**	**Product E**	**Product F**
Actual Hg in ng/ml	516.28	1315.17	6651.77	5383.77	477.21	2741.13
Actual Hg in ng/50ml	25814.00	65758.50	332588.50	269188.50	23860.50	137056.50
Hg in 1 kg of Product (ng/kg)	26.01E6	65.86E6	329.88E6	267.53E6	23.78E6	136.39E6
Hg in Product (ppm)	**26.01**	**65.86**	**329.88**	**267.53**	**23.78**	**136.39**

APPENDIX 4: TOTAL MERCURY ANALYZER, PRSS



APPENDIX 5: CALIBRATION CURVE OF TOTAL MERCURY ANALYZER



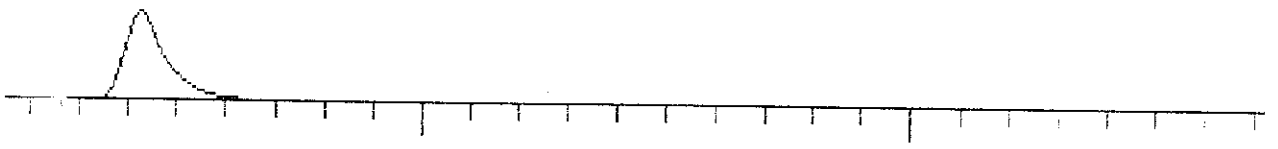


APPENDIX 6: EXPERIMENTAL VOLATOGRAMS

ST Ref FNLCR6 Run 1

aseLine: 0.3%

Peak Area: 159.5xsec
Peak Height: 21.3%
Retention:13.0s



00 Filter 4 Time - 272secs Signal> 0.200%

Printed from TouchStone 9 Sep 04

LCR6 Run 1 Peak Height= 21.3 Peak Area= 159.5 Retention:13.0s
NLCR6 21.3 14.05ug/m3 L 0.000ug/m3 15:05

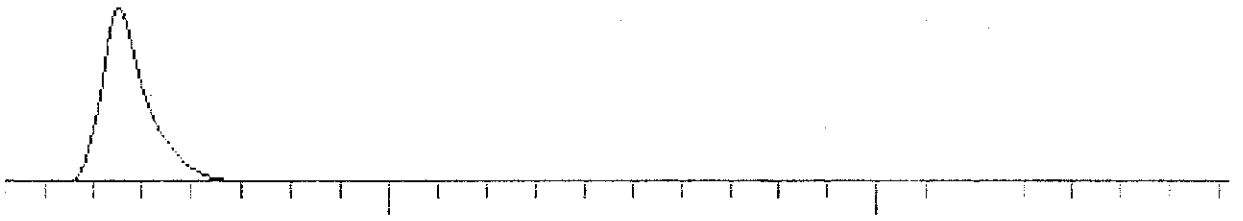
Ref PFF4 Run 1

Peak Area: 352.6%sec

Line: -9.2%

Peak Height: 43.2%

Retention:15.5s



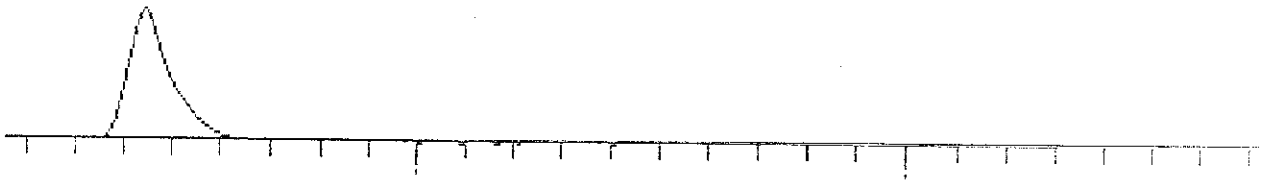
Filter 4 Time - 272secs Signal) -8.38%

Printed from TouchStone 9 Sep 04

:T Ref PFF5 Run 1

.seLine: -0.5%

Peak Area: 252.6%s.
Peak Height: 32.5%
Retention:15.0s



00 Filter 4 Time - 272secs Signal> -0.70%

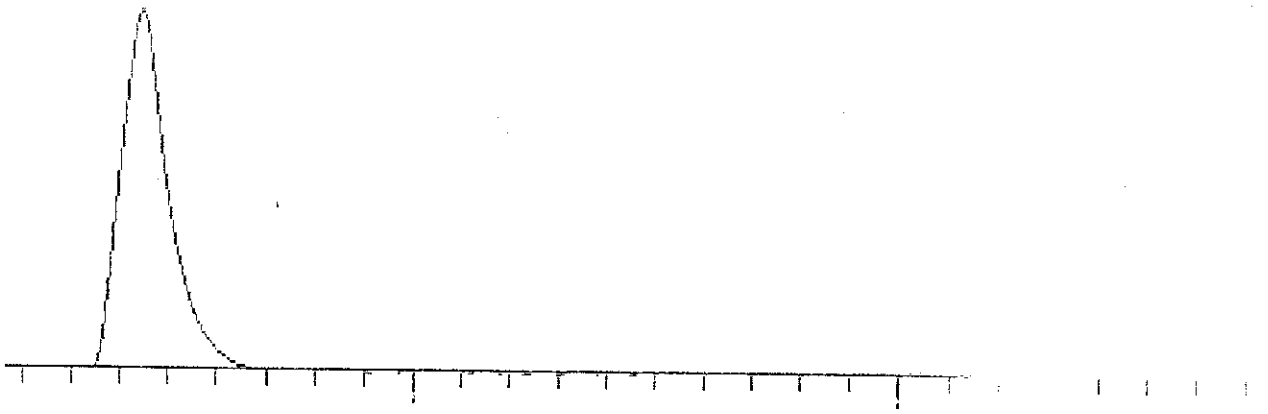
Printed from TouchStone 9 Sep 04

F5 Run	1	Peak Height=	32.5	Peak Area=	252.6	Retention:15.0s
PFF5	32.5	22.16ug/m3	1	0.000ug/m3	16:10	
NK Run	1	Peak Height=	0.8	Peak Area=	5.2	Retention:10.0s
BLANK	0.8	0.000ug/m3	1	0.000ug/m3	16:18	

T Ref 1PFF Run 1

seLine: 0.0%

Peak Area: 717.0%sec
Peak Height: 88.3%
Retention:14.0s



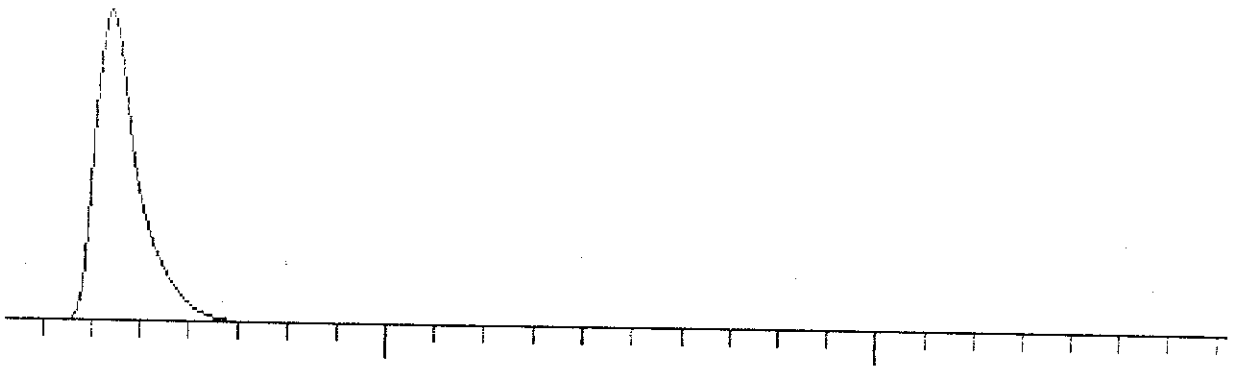
00 Filter 4 Time - 272secs Signal> -0.60%

Printed from TouchStone 9 Sep 04

FF Run	1	Peak Height=	88.3	Peak Area=	717.0	Retention:14.0s
1PFF	88.3	32.50ug m3	1	0.000ug/m3	17:07	
FF Run	1	Peak Height=	57.0	Peak Area=	417.8	Retention:14.5s
2PFF	57.0	29.88ug m3	1	0.000ug/m3	17:15	
NK Run	1	Peak Height=	0.8	Peak Area=	8.9	Retention:15.0s
LANK	0.8	0.000ug m3	1	0.000ug/m3	17:27	

eLine: -0.1%

Peak Area: 585.8%sec
Peak Height: 76.7%
Retention:13.5s



Filter 4 Time - 272secs Signal> -0.38%

Printed from TouchStone 9 Sep 04

3 Run 1 Peak Height= 76.7 Peak Area= 585.0 Retention:13.5s
FI3 76.7 64.09ug/m3 1 0.000ug/m3 18:38

Ref SAFI4 Run 1

Line: 0.2%

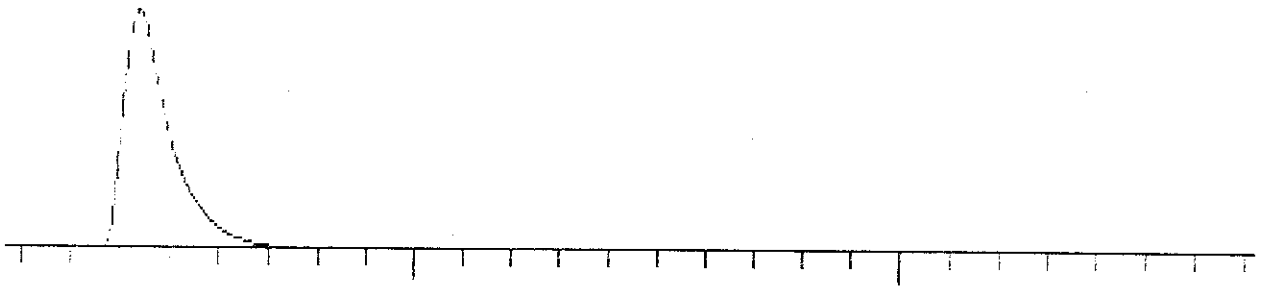
Peak Area: 845.8%sec
Peak Height: 106.2%
Retention:14.8s

weight 102

T Ref SAFI6 Run 1

seLine: -0.0%

Peak Area: 486.1%sec
Peak Height: 58.8%
Retention:14.5s



)0 Filter 4 Time - 272secs Signal> -0.10%

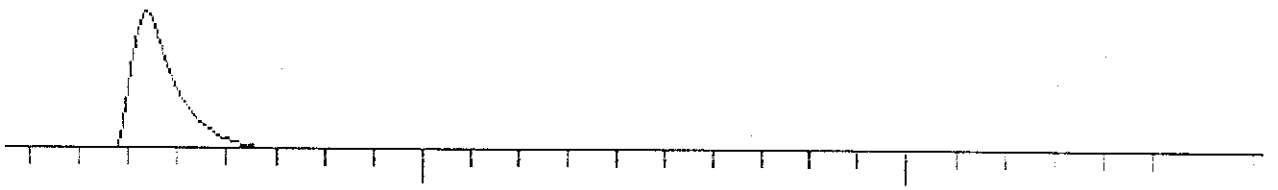
Printed from TouchStone 10 Sep 04

?I6 Run	1	Peak Height=	58.8	Peak Area=	486.1	Retention:	14.5s
SAFI6	58.8	75.86ug/m3	1	0.000ug/m3	11:58		
NK Run	1	Peak Height=	0.5	Peak Area=	4.5	Retention:	14.5s
BLNK	0.5	0.393ug/m3	1	0.000ug/m3	12:05		

:T Ref SAFI7 Run 1

.seLine: -0.3%

Peak Area: 272.6%sec
Peak Height: 34.1%
Retention:13.5s



00 Filter 4 Time - 272secs Signal> -0.30%

Printed from TouchStone 10 Sep 04

FI7 Run	1	Peak Height=	34.1	Peak Area=	272.6	Retention:13.5s
SAFI7	34.1	59.65ug/m3	1	0.000ug/m3	12:14	
NK Run	1	Peak Height=	0.3	Peak Area=	0.7	Retention:21.0s
BLNK	0.3	0.131ug/m3	1	0.000ug/m3	12:21	

T Ref SAFI8 Run 1

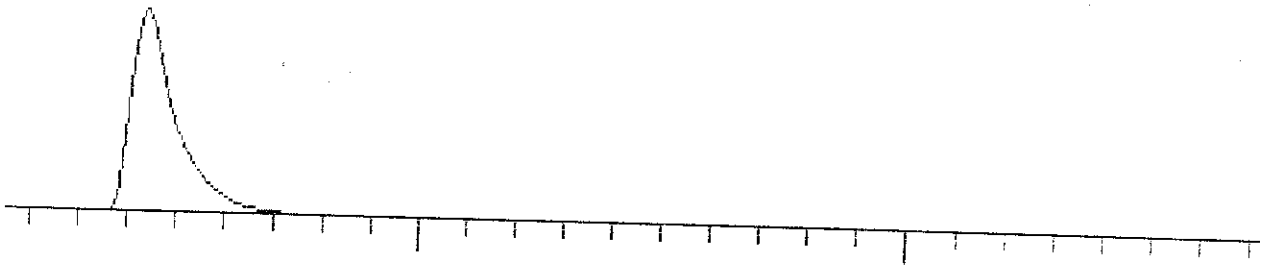
seLine: -0.3%

Peak Area: 373.2%sec
Peak Height: 45.8%
Retention:14.0s

IGT Ref SAFI9 Run 1

aseLine: 0.2%

Peak Area: 402.2%sec
Peak Height: 49.7%
Retention:14.0s



30 Filter 4 Time - 272secs Signal> 0.300%

Printed from TouchStone 10 Sep 04

F19 Run	1	Peak Height=	49.7	Peak Area=	402.2	Retention:14.0s
SAFI9	49.7	72.46ug/m3	1	0.000ug/m3	12:40	
NK Run	1	Peak Height=	0.2	Peak Area=	1.4	Retention: 9.0s
BLNK	0.2	0.003ug/m3	1	0.000ug/m3	12:46	

- END OF REPORT -