CHAPTER II

LITERATURE REVIEW

Human skin

Skin is the outermost layer and the largest organ in terms of weight and surface area in human body. The area of skin covers approximately 16,000 cm^2 in adult and 8% estimate of the human body weight (Igarashi, et al., 2007). Skin composes of a three main multiple layers including epidermis, dermis and subcutaneous layer. Skin has a complex structure (Figure 1) which provides many functions and useful properties.

Function of skin

1. Act as a protective barrier from harmful chemical, bacteria, viruses, and ultraviolet light
2. Prevent water loss
3. Regulate temperature of the body by blood flow and evaporation of sweat
4. Metabolite and synthesize substances
5. Act as a sensory organ

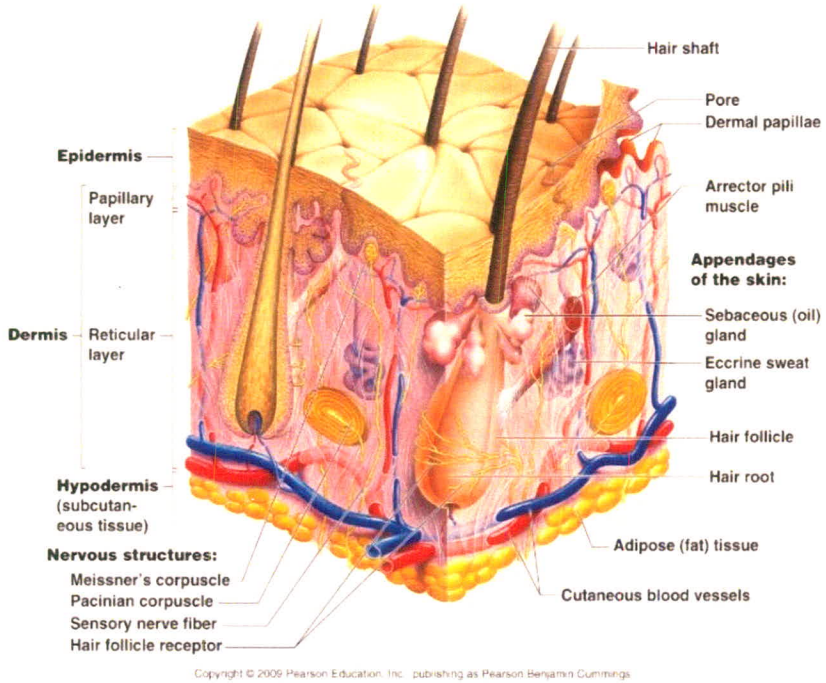


Figure 1 The structure of skin

Source: Biological pictures, 2014

Skin layers

Epidermis

The epidermis is the most superficial layer of the skin which has a variety of thickness depending on a part of body. The average thickness is approximately 0.2 mm. Epidermis is divided into 5 sublayers including stratum basal, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum.

Stratum basal (basal layer or stratum germinativum) is the deepest layer of epidermis. There are many abundant cells such as keratinocytes, melanocytes, including immune response cells called Langerhans cells that are also found in this layer. The cells are produced and move upward to upper layer to change their structure and function. Melanocyte cells produce melanin for light absorbing pigments which are responsible for skin color including tan, brown, or yellow and help to protect the deeper layers from the harmful effects of sun exposure. In addition, the prickle cells which composed of

bipolar lipids to prevent evaporation of water and allow the skin to retain moisture are typically produced in this layer.

Stratum spinosum (prickle cell layer) is located above the stratum basal. The local cells in this layer are spiny cells. It is a specialized cell for cell-to-cell adhesion which connects together through the spiny projections. The keratinocytes development process called "Keratinization" occurs in this layer. The cells start to synthesize keratins which are a major protein of skin structure. Thereafter, the proteins moved through the stratum spinosum and move upward to upper layer.

Stratum granulosum (granular cell layer) is the middle layer of the epidermis. The keratinocytes from the stratum spinosum are pushed up to this layer. The cells in this layer function by change their structure to flat and become less moisture. Finally, it turns into dead cells and moves upward to outer layer.

Stratum lucidum (clear layer) is the layer of flattened dead cells which functions the same as the stratum granulosum layer. This layer canbe found in only skin soles and palms.

Stratum corneum (horny cell layer) is the outermost layer of the epidermis. It consists of dried dead cells called horny cells or corneocytes that are filled with keratin fibers and surrounded by intercellular lipid.

In epidermis layer, there is a metabolically active tissue called "turnover process". During the turnover process, the keratinocytes in stratum basal or basal layer move upward to upper layer. During moving, the keratinocytes change their structure into flat shape and physiological function. These cells lose their activity and become dead cells. The turnover process spends about 28 days. However, the time varies depending on many factors such as age, individual skin properties, and environment.

Dermis

The dermis is located between the epidermis and subcutaneous. Its thickness is approximately 1 to 4 mm. This layer is connected with the nerve ending, capillaries, sweat and sebaceous glands, and hair follicle. A type of common cells called fibroblasts which synthesize the connective tissue including collagen and elastin fiber are also found in this layer.

Subcutaneous

The subcutaneous or hypodermis is the lowermost layer of skin with a thickness of 4-9 mm in average. This layer constitutes of flat cells which a shock absorber and energy storage. In addition, there are many cells founded in this layer such as fibroblasts, adipose cells, and macrophages.

Ultraviolet radiation

Solar energy is the significant primary source which supports almost all the living on the earth. The energy can be delivered in the form of electromagnetic wave, heat and light which is visible and invisible. Ultraviolet (UV) radiation is one of the essential visible lights in the range of wavelength between 200 and 400 nm. UV radiation is subdivided into three bands from the longer to the shorter wavelength as follows (Pathak, 1997):

UVA (black light radiation, long-wavelength UV radiation, near UV radiation) has the longest wavelength, between 320 and 400 nm. UVA radiation reaches the earth approximately 95% and can penetrate deeper into both epidermis and dermis layer of human skin (Figure 2). In the dermis layer, UVA can stimulate the formation of melanin and produce a tanning effect. In addition, excessive UVA exposure may also cause photo aging and damage to the skin's immune system.

UVB (sunburn radiation, middle-wavelength UV radiation) has a shorter wavelength, between 290 and 320 nm. About 5% remaining of the UVB radiation reaches the earth which can penetrate into epidermis of human skin (Figure 2). UVB radiation causes sunburn and some types of skin cancer.

UVC (germicidal radiation, short-wavelength UV radiation, far UV radiation) is the shortest wavelength of UV radiation and the highest energy, between 200 and 290 nm. The UVC radiation can damage collagen fibers, causes an aging and accelerates skin cancer. Fortunately, UVC is absorbed by the ozone layer. Therefore, UVC radiation does not reach the earth's surface.

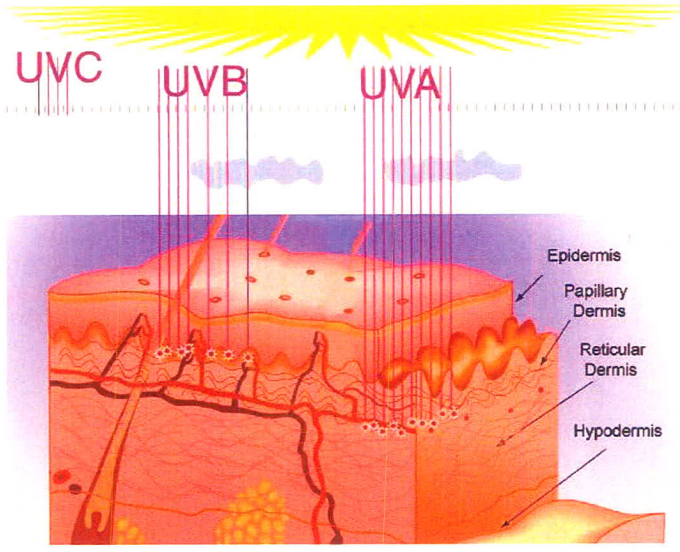


Figure 2 UV radiation skin penetrations

Source: Melbourne Laser and Aesthetic Centre, 2013

Effects of UV radiation

Although excessive exposure to UV radiation can cause harmful effects, complete avoidance of UV radiation does not always provide a good advantage. Therefore, it is necessary to consider both benefits and risks of UV radiation.

Beneficial effects of UV radiation (Shivani, et al., 2010)

1. Increase blood circulation.
2. Increase formation of hemoglobin.
3. Help to reduce blood pressure.
4. Increase vitamin D production.
5. Help to enhance calcium absorption from intestine.
6. Help to produce melanin and cause thickening of skin, which presents natural protective mechanism against sunburn.

Adverse effects of UV radiation

Even UV radiation provides many benefits to human. The excessive exposure to UV radiation may also cause many adverse effects, depending on duration, UV exposure dose and responsibility of individual person.

1. Sunburn

Sunburn is a short term effect of excessive UV exposure. The skin responds to an excessive UV exposure by releasing chemicals those dialate blood vessels, leading to fluid leakage and inflammation. Therefore, the commond symtoms are redness, swelling, feeling of heat, mild to severe pain, tender to the touch and blisters in severe cases. These signs appear 1-24 hours after UV exposure and subside within a couple of days. As stated above, UVB radiation possesses higher energy than UVA. Therefore, UVB radiation can cause sunburn and induce erythema more severe than UVA (Kaidbey and Klingman, 1979). Repeated sunburn may cause premature wrinkle and risk of skin cancer in the long-term.

2. Tanning

Tanning is the one type of adverse effect after exposure to UV radiation. It is involves the dysfunction of melanin synthesis. Melanins are a type of pigment which are produced from melanocyte cells. This melanin production is activated when our skin is exposed to UV radiation. The immoderate dose of UV can induce the excessive pigment synthesis called hyperpigmentation and change skin to darker tone (Mcguire, et. al, 1992).

3. Photoaging

Photoaging is a chronic effect on human skin following long-term exposure to UV radiation. It usually causes by UVA rather than UVB due to the UVA radiation can penetrate deeper into the dermis layer. The excessive exposure to UVA can make the basal layer becomes thinner and loses ability to retain water. It produces an effect on degradation of connective tissue fiber in dermis layer including collagen and elastin, resulting in incorrectly rebuilt skin and decrease smoothness of skin (Klingman and Klingman, 1997).

4. Photosensitivity

Photosensitivity is the broad term used to mention adversed responses to UV radiation including phototoxic and photoallergic. Phototoxicity is generally involved inflammatory reaction which expresses by erythema and edema followed by hyperpigmentation whereas photoallergy involves with immunological reaction. The differences between a phototoxic and a photoallergic reaction are shown in Table 1.

Table 1 Distinction between phototoxicity and photoallergy (Lugovic, et al., 2007)

Feature	Phototoxic reaction	Photoallergic reaction
Incidence	High (more common)	Low (less common)
Amount of agent required for photosensitivity	Large	Small
Mechanisms	No immune reactions, light-activated cell membrane compounds and DNA	Immunologically mediated cell-mediated immune responses (type IV) to a light-activated compound
Onset of reaction after exposureto agent and light	Minutes to hours	24–72 hours
Distribution	Sun-exposed skin only	Sun-exposed skin, may spread to unexposed areas
Clinical characteristics	Exaggerated sunburn	Dermatitis, photoallergen applied topically eczematous morphology; photoallergen systemically drug eruption

In addition, excessive UV exposure can also cause other effects such as malignant melanoma, skin cancer (photocarcinogenesis), alteration of local and systemic immune suppression (Pathak, 1997).

Sunscreen

Sunscreen, a cosmetic product containing UV filters, is recommended by dermatologist to use in daily life in order to protect human skin from the UV radiation. It is usually used in form of creams, lotions, sprays or solutions. In 1928, the world's first sunscreen was developed in the United States in the form of emulsion containing benzoyl salicylates and benzoyl cinnamate (Patini, 1988). In the early 1930s, the

sunscreen solution of phenyl salicylate was occurred in the Australian commercial market. Later, the new trends were continuously developed and *p*-aminobenzoic acid (PABA) became a most popular UV filter in sunscreen since 1943s. Initially, most of sunscreen was purposed to protect skin from the UVB radiation which can induce acute sunburn. Subsequently, there are many researches published the harmful effect of UVA induced skin. Therefore, both UVB and UVA protection are also significantly concerned in the production of sunscreen products nowadays.

UV filters

UV filters are substances which are used to protect or limit damage of skin from UV exposure and function by absorbing or reflecting the UV radiation. Base on the mode of action, UV filters can be classified into two groups.

1. Chemical UV filters

Chemical UV filters or organic UV filters are synthetic substances which are generally aromatic compounds conjugated with a carbonyl group. The chemical filters function by absorbing the harmful UV radiation. Following energy absorption, the molecules of chemical filters are excited from ground state to excited state and returns to ground state by emitting the healthful longer wavelength which is a lower energy than the initial (Shaath, 1997). The first patent chemical UV filter was *p*-aminobenzoic acid (PABA) in 1943s. Then, the other new UV filters has been introduced and continuously gained popularity. Consequently, there has many publications report on adverse effects of some UV filters. A popular PABA was the one of the filters banned in 2008s since there has been reported of side effects in allergic and irritation. In addition, the reports suggested that PABA was an unsafe substance and was not approved to be used in European, Australian/New Zealand, Canada and Asian including Thailand. For other substances, there has been continuously published their side effect, so that the safety has recently been questioned. Presently, there are 26 chemical substances which are approved by Thai FDA and allowed to be used in sunscreen products. The substances are listed in Table 2.

2. Physical UV filters

Physical UV filters or inorganic UV filters are opaque substances which function by scattering or reflecting the UV radiation. It provides a broad spectrum protection against both UVA and UVB. There are several forms, for example, titanium

dioxide, zinc oxide, other metal oxides, kaolin, ichthammol, red veterinary petrolatum, talcum, calamide, etc (Lowe and Friedlander, 1997). According to Thai FDA guidelines, there are currently two substances, titanium dioxide and zinc oxide, that are allowed to be used as physical UV filters in sunscreen products. In the past, conventional physical UV filters have large particle size and thus give undesired whitening effect, staining clothing and do not easily wash off. Currently, the micronized physical UV filters has been recommended. The micronized filters are the preparation of ultrafine ground materials having particle size less than 200 nanometers and they are more transparent on skin. The physical UV filters are generally recommended in sunscreen for kid and individuals who have unusual sensitivity skin, due to the acceptance of pretty safety which does not penetrate into human skin and does not provide a free radical. However, there are many concerns with the safety of nano-sized physical UV filters.

Table 2 The chemical UV filters approved to be used in sunscreen product by Thai FDA (Ministry of Public Health, 2012)

UVB filters	UVA filters	UVB&UVA filters
1. 3-Benzylidene camphor	1. Benzophenone 3	1. Bis-ethylhexyloxyphenol methoxy phenoltriazine
2. 4-Methylbenzylidene camphor	2. Benzophenone-4	2. Drometrizoletrisiloxane
3. Benzylidene camphor sulfonic acid	3. Butyl methoxydibenzoyl-methane	3. Methylene bis-benzo-triazolyltetramethyl-butylphenol
4. Camphor benzalkonium	4. Diethylaminohydroxy-benzoyl hexyl benzoate	
5. Diethylhexyl-butamidotriazone	5. Menthylanthranilate	
6. Dimethicodiethylbenzal malonate	6. Terephthalylidene-dicamphor sulfonic acid	
7. Ethylhexyl dimethyl PABA		

Table 2 (Cont.)

UVB filters	UVA filters	UVB&UVA filters
8. Ethylhexyl methoxy cinnamate		
9. Ethylhexyl salicylate		
10. Ethylhexyl triazone		
11. Homosalate		
12. Isoamyl *p*-methoxy cinnamate methosulfate		
13. Monosodium salt of 2-2'-bis-(1,4-phenylene)-*1H*-benzimidazole-4,6-disulfonic acid		
14. Octocrylene		
15. PEG-25 PABA		
16. Phenylbenzimidazole sulfonic		
17. Polyacrylamidomethyl-benzylidene camphor		

Factors determining efficacy of sunscreen products

The efficacy of UV protection in sunscreen products indicates by many parameters including UVB protection, UVA protection and water resistant.

1. UVB protection

SPF (Sun Protection Factor) value labeled on sunscreen products indicates the UVB protection efficacy. This number suggests how much longer a person can stay in the sun without getting burn. The SPF is calculated by the ratio of the minimal dose of solar radiation producing minimum erythema on the skin that is applied with sunscreen compared with unapplied skin. For example, if it takes 20 minutes for a person to get burn, a sunscreen product with SPF 15 will let a person stay in the sun 15 times longer (i.e. 300 min) without getting burn. Based on global norms, SPF values can be categorized as low, medium, high and maximum or very high as shown in Table 3.

Table 3 Classification of Sun Protection Factor (SPF)

US FDA	COLIPA/JCIA	AS/NZS	Thai FDA
2 - 15 Low	6,10 Low	4,6,8,10 Low	≥6 to <15 Low
15 - 30 Medium	15,20,25 Medium	15,20,25 Medium	≥15 to <30 Medium
30 - 50 High	30,50 High	30,40,50 High	≥30 to <50 High
50+ Maximum	50+ Very high	50+ Very high	50+ Very high

2. UVA protection

On the label of sunscreen products, there are different signs which indicate the UVA protection efficacy, depending on international agencies.

In US, a number or level of UVA protection efficacy were not regulated. However, the UVA protection labeled on sunscreen products indicates by the word "broad spectrum" which refers to both UVA and UVB radiation.

In EU, the UVA protection efficacy is shown as the UVA protection logo but not indicates the protection level. The UVA protection claimed on sunscreen products must be proven by results on a critical wavelength test which required at the minimum 1:3 ratio of UVA protection to UVB protection. The logo of UVA protection regulated by COLIPA is shown in Figure 3.

Figure 3 The UVA protection logo on sunscreen product label

Source: Wikimedia Commons, 2014

In UK, a level of UVA protection indicates by Boots star rating system which obtained by measuring the absorption of UVA and UVB through the sunscreen applied on a PMMA plate before and after exposure to a fixed UV dose. The value is calculated by the ratio of mean UVA absorbance and mean UVB absorbance. The rating criteria of Boots star system is shown in Table 4. The Boots star rating logo on sunscreen product label is shown in Figure 4.

Table 4 Boots star rating system in 2011 revision

		Initial exposure Mean UVA:UV B ratio			
		0.0 to 0.59	0.6 to 0.79	0.8 to 0.89	0.9 and over
Post exposure Mean UVA:UV B ratio	0.0 to 0.56	No rating	No rating	No rating	No rating
	0.57 to 0.75	No rating	★★★	★★★	★★★
	0.76 to 0.85	No rating	★★★	★★★★	★★★★
	0.86 and over	No rating	★★★	★★★★	★★★★★

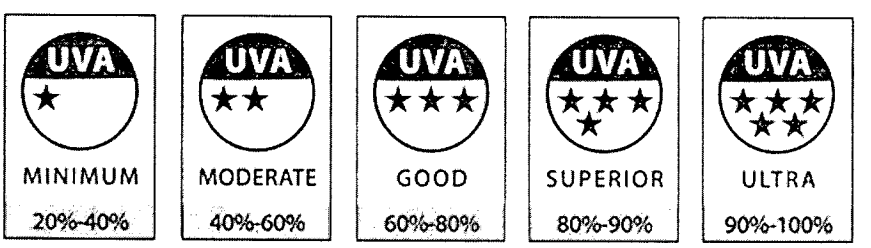


Figure 4 The Boots star rating logo on the label of sunscreen product

Source: Uvistat, 2014

In Japan, the efficacy of UVA protection of sunscreen products indicates by PA rating which regulated by the Japan Cosmetic Industry Association (JCIA). The value is obtained from an *in vivo* Persistent Pigment Darkening (PPD) or Protection of factor UVA (PFA or UVAPF) method. Similar to SPF, UVAPF is calculated by the ratio of the minimal dose of UVA radiation producing persistent pigment darkening on sunscreen protected skin compared with unprotected skin. The Protection Grade of UVA (PA) system is tabulated in Table 5.

Table 5 PA rating system

UVAPF	UVA protection level	Rating
2 to less than 4	Low	PA+
4 to less than 8	Medium	PA++
8 to less than 16	High	PA+++
16 and above	Very high	PA++++

3. Water resistant

Water resistant indicates how well a sunscreen product sticks onto skin after swimming, exercise and sweating situation. The label claim of "water resistant" must specify how long the sunscreen can withstand these situations, i.e. "water resistant (40 minutes)" or "very water resistant (80 minutes)". US FDA and COLIPA now bans the terms "sunblock," "waterproof," or "sweatproof" on the product label.

Sunscreen regulations

Most of commercially available sunscreen products contain a combination of various UV filters that absorb different region of UV radiation in order to achieve high protection of UV radiation. Therefore, all international agencies as well as Thai Food Drug Administration (Thai FDA) have regulated the maximum concentration of UV filters allowed to be used in sunscreen products. There are 28 substances which approved by Thai FDA to be used in sunscreen products as shows in Table 6.

Table 6 The UV filters approved by Thai FDA with a maximum concentration allowed to be used in sunscreen products (Ministry of Public Health, 2012)

	UV filters		CAS No.	Maximum concentration (%)
	Chemical name	Common name		
1	*N,N,N*-Trimethyl-4-(2-oxoborn-3-ylidenemethyl) anilinium methyl sulfate	Camphor benzalkonium-methosulfate	52793-97-2	6
2	Homosalate	Homosalate	118-56-9	10
3	Oxybenzone	Benzophenone 3	131-57-7	10
4	2-Phenylbenzi midazole-5-sulfonic acid and its potassium, sodium and triethanolamine salts	1.Phenylbenzimidazole sulfonic acid 2.Sodium phenylbenzi-midazolesulfonate 3.TEA phenylbenzi-midazolesulfonate	27503-81-7 5997-53-5 73705-00-7	8*
5	3,3'-(1,4-Phenylenedimethylene)- bis(7,7-dimethyl-2-oxobicyclo-[2,2,1]hept-1-ylmethanesulfonic acid) and its salts	Terephthalylidenedicam-phor Sulfonic acid	90457-82-2	10*
6	1-(4-*tert* - Butylphenyl)-3-(4-methoxyphenyl) propane-1,3-dione	Butyl methoxydibenzoyl Methane (Avobenzone)	70356-09-1	5

Table 6 (Cont.)

	UV filters		CAS No.	Maximum concentration (%)
	Chemical name	**Common name**		
7	alpha-(2-Oxoborn-3-ylidene) toluene-4-sulfonic acid and its salts	Benzylidene camphor sulfonic acid	56039-58-8	6*
8	2-Cyano-3,3-diphenyl acrylic acid, 2-ethylhexyl ester	Octocrylene	6197-30-4	10*
9	Polymer of *N*-{(2 and 4)-[(2-oxoborn-3-ylidene) methyl] benzyl} acrylamide	Polyacrylamidomethyl benzylidene camphor	113783-61-2	6
10	Octylmethoxy-cinnamate	Ethylhexylmethoxy-cinnamate	5466-77-3	10
11	Ethoxylated ethyl-4-aminobenzoate	PEG-25 PABA	116242-27-4	10
12	Isopentyl-4-methoxycinnamate	Isoamyl*p*-methoxy cinnamate	71671-10-2	10
13	2,4,6-Trianilino-(*p*-carbo-2'-ethylhexyl-1' oxy)-1,3,5-triazine	1. Octyl triazone 2. Ethylhexyl triazone	88122-99-0	5

Table 6 (Cont.)

	UV filters		CAS No.	Maximum concentration (%)
	Chemical name	**Common name**		
14	Phenol,2-(2H-benzotriazol-2-yl)-4-methyl-6-(2-methyl-3-(1,3,3,3-tetramethyl-1-(trimethylsilyl)oxy)-disiloxanyl)propyl)	Drometrizoletri-siloxane	155633-54-8	15
15	Benzoic acid, 4,4-[[6-[[(1,1-dimethylethyl) amino]carbonyl]phenyl]amino] 1,3,5-triazine-2,4-diyl]diimino)bis-,bis-(2-ethylhexyl)] ester	Diethylhexylbutamido triazone	154702-15-5	10
16	3-(4'-Methylbenzylidene)-d-1 camphor	4-Methylbenzylidene camphor	36861-47-9	4
17	3-Benzylidene camphor		15087-24-8	2
18	2-Ethylhexyl salicylate	1. Octyl salicylate 2. Ethylhexyl salicylate	118-60-5	5

Table 6 (Cont.)

	UV filters		CAS No.	Maximum concentration (%)
	Chemical name	**Common name**		
19	4-Dimethyl-amino-benzoate of ethyl-2-hexyl	1. Octyl dimethyl PABA 2. Ethylhexyl dimethyl PABA 3. Padimade O	21245-02-3	8
20	2-Hydroxy-4-methoxy benzophenone-5-sulfonic acid and its sodium salt	Benzophenone 4	4065-45-6	5*
21	2,2'-Methylene-bis-6-(2H-benzotriazol-2yl)-4-(tetramethyl-butyl)-1,1,3,3-phenol	Tinosorb®M	103597-45-1	10
22	Monosodiumsalt of2-2'-bis-(1,4-phenylene)-*1H*-benzimidazole-4,6-disulfonicacid		180898-37-7	10*
23	(1,3,5)-Triazine-2,4-bis((4-(2-ethyl-hexyloxy)-2-hydroxy)-phenyl)-6-(4-methoxyphenyl)	Tinosorb®S	187393-00-6	10

Table 6 (Cont.)

	UV filters		CAS No.	Maximum concentration (%)
	Chemical name	Common name		
24	Dimethicodiethylbenz almalonate		207574-74-1	10
25	Titanium dioxide		13463-67-7	25
26	Benzoic acid, 2-[-4-(diethylamino)-2-hydroxybenzoyl]-, hexylester	Diethylaminohydroxy benzoyl hexyl benzoate	302776-68-7	10
27	Menthylanthranilate		134-09-8	5
28	Zinc oxide		1314-13-2	25

* calculated as acid form

Determination of UV filters in sunscreen products

According to the regulation of maximum permit amount used in sunscreen products in many international regulatory agencies, there are no official analytical methods for determination of UV filters in sunscreen products. Up to now, many methods used to assay UV filters have been published. The methods include spectroscopy and chromatography.

Spectroscopy

Spectroscopy is a technique that measures light which is emitted, absorbed, or scattered by materials. Various spectroscopic techniques are used to analyze UV filters including ultraviolet spectroscopy (UV), infared spectroscopy (IR), mass spectrometry (MS), and nuclear magnetic resonance (NMR).

Ultraviolet (UV) spectroscopy is a technique that the molecules or atoms of substance has electronic transitions which is excited by UV-VIS light (200-800 nm) from ground state to excited state, an absorbance or a transmittance value is measured between transition process. In 2006, Moeur, et al. (2006) used UV spectroscopy couple

with diode-array to quantitate ethylhexyl methoxycinnamate in sunscreen lotion by using 2-propanol as a solvent. However, this technique works well on liquids or solutions conversely not suitable for a suspension of solid particles due to the substances can scatter the light more than absorb and the result will not be accurate.

Mass spectrometry (MS) is an analytical technique which the chemical substances are generated the gaseous ions in electric and magnetic fields. This technique measures mass to charge ratio (m/e) of ions and is used to elucidate the chemical structure of a molecule. It has a high sensitivity over other techniques. It is rarely use for quantitative analysis but often uses as a detector coupled with other instruments such as gas chromatography. However, this technique is very complex and expensive for a maintenance.

Nuclear magnetic resonance (NMR) is one of the spectroscopic technique which involves electrically charge of nuclei and specified spin property of different substances. In 1996, Mori, et al. (1996) used the NMR for determination of five UV filters which were benzophenone-1, benzophenone-3, butyl methoxy dibenzoylmethane, ethylhexyl dimethyl PABA and ethylhexyl methoxycinnamate. However, disadvantages of NMR are very expensive for maintenance and require complex skill to operate.

Chromatography

Chromatography is the separation technique which separates a complex mixture by passing the mixture through a stationary phase. The principle of separation is the distribution of a solute between the mobile and stationary phases. The chromatography techniques can be categorized in several types, for example, thin layer chromatography (TLC), gas chromatography (GC) and high performance liquid chromatography (HPLC).

Thin layer chromatography (TLC) is the simple and rapid technique for determination of UV filters. The separation is performed by spotting the sample on the plate coated with absorbent material (stationary phase) and the solvent (mobile phase) will travel up the plate and pass over the spot by capillary force. The compounds in the mixture sample move with solvent at different rates depending on their solubility in these two phases.

Gas chromatography (GC) is the analytical technique which uses the carrier gas as a mobile phase passing through the column or stationary phase. The sample is vaporized in a hot injection chamber. The vaporized sample is moving through a column of adsorbent material (stationary phase) by a stream of carrier gas (mobile phase) such as nitrogen or helium. Then, the signal is detected and monitored in response units. It usually coupled with mass spectrometry in an analysis. In 1990, Ikeda, et al. (1990) used gas chromatography coupled with mass spectrometry for determination of benzophenone-3, butylmethoxy dibenzoylmethane, ethylhexyl dimethyl PABA, ethylhexyl methoxycinnamate, isopropyl dibenzoyl methane. However, the limitation of gas chromatography is that the samples have to be volatile.

High performance liquid chromatography (HPLC) is one type of liquid chromatography which is widely used in qualitative and quantitative analysis. This technique was gained attention in this study because it is a rapid analytical technique as well as a high degree of resolution, selectivity and reproducible. It is effective for routine analysis and convenient on working due to its automated operation system. In addition, it is available in most of cosmetic companies. Therefore, HPLC was the chosen technique for determination of UV filters in sunscreen products in this study.

High performance liquid chromatography; HPLC

Principle of HPLC

High performance liquid chromatography is one type of the liquid chromatography. The separation is occurred in a column containing various stationary phases under high pressure. The mechanisms of separation depend on the types of stationary phase and mobile phase used in an assay. Compounds are separated on column and subsequently detected by the detector corresponding to analytical peaks in the chromatogram. The separation bases on whether the component has an affinity for the stationary or the mobile phase. Components which have high affinity for the mobile phase are eluted from the column faster. On the other hands, components which have high affinity for the stationary phase are eluted from the column slower. The HPLC can be used in quantitative and qualitative analysis in many applications. The advantages are rapid, precise, high sensitivity, high resolution, quantitative sample recovery and

automated operation. However, the limitations are irreversibly adsorbed compounds probably not be detected and no universal detector.

Mode of HPLC

There are many separation modes of HPLC including normal phase chromatography (NPC), reversed-phase chromatography (RPC), ion-exchange chromatography (IEC) and size-exclusion chromatography (SEC), etc. Among these techniques, reversed-phase chromatography is the most widely used mode of HPLC. This mode offers a wide variety to cover a broad range of polar selectivity and works well for analysis of polar (water-soluble), medium polarity and some non-polar compounds. In addition, it can be applied to analyze ionic substances by using ion-suppression or ion-paring technique. Therefore, this mode is chosen in the analysis of UV filters in this study.

Reversed-phase HPLC employs a non-polar stationary phase and typically polar mobile phase. The typical reversed-phase column contains a small packing material with diameters 3-10 μm. The general characteristics of analytical column are 50-250 mm long with an internal diameter of 2.0-4.6 mm. Bonded silica is usually used as packing materials in the reversed-phase column. The silanol groups of the silica are substituted with a chemically-bonded layer of some type of non-polar molecule such as C8 (octylsilane) or C18 (octadecylsilane) to provide non-polar property. The separation relies upon a partition coefficient of the substances between the mobile phase and the stationary phase. The factors affecting the separation are the polarity properties of substance, elution strength of organic solvents used in the mobile phase as well as pH of the mobile phase, column temperature, and the flow rate of mobile phase. The polarity affects the elution order which in reversed-phase chromatography is polar first and non-polar later because of the stronger interaction between the non-polar compound and the hydrophobic stationary phase. As well as the elution strength of organic solvent used in mobile phase, non-polar solvent has higher elution strength resulting in faster elution than polar solvent. With respect to the effects of column temperature, the higher temperature can reduce the viscosity of the mobile phase and resulting in a reduction of column back pressure. The molecules can move easier in higher temperature than the lower temperature condition. Therefore, the total analysis time is generally decreased when increasing column temperature. The pH of mobile phase affects the separation by

involving with the analyte ionization resulting in the interaction of analytes with the stationary phase. At the low pH, substances with the high pK_a is well ionized and carries a positive charge and behaves as an hydrophobic molecule which can be more retained than at high pH in reverse phase. However, pH adjusting is a considerable method used to solve a poor resolution and tailing problems. In addition, the flow rate is one of the factors affecting the separation of substances. Increasing flow rate causes less interaction between substances and stationary phase leading to a shorter total analysis time as well as a reduction of peak resolution.

Types of elution

1. Isocratic elution mode

An isocratic mode is the basic mode of HPLC separation which is commonly used in many laboratories. This mode uses a constant ratio of solvent as a mobile phase to elute substance passing through the column. It is suitable for analyzing simple substances, and it provides a stable baseline as well as unvarying response factor. However, the limitation of this mode is that it cannot elute the complicate substance (e.g. a wide range of polarity) and may cause a long-time analysis.

2. Gradient elution mode

Gradient elution is the modified mode which improves separation of complex substances by changing composition and ratio of the composition of the mobile phase during the system operation. The elution strength of the mobile phase can be adjusted between the sample analyses. Therefore, the gradient mode is expected to provide a short-time analysis and an improved resolution as compared to the isocratic mode. It is suitable for complex substance or multiple analyzes of diverse polarity which provides better resolution and sensitivity of peak. However, this mode requires more complex instrument and complicate development than the isocratic mode.

HPLC systems

HPLC system consists of a mobile phase reservoir, a pump, an injector or auto sampler, a column, a detector and a display system. A high pressure pump generates a specific and constant flow rate of the mobile phase expressed in milliliters per minute (mL/min). A substance is introduced into the HPLC systems by an injector or auto sampler. With respect to the separation, the substance is introduced into the continuously flowing liquid mobile phase that carries substances through the column

under high pressure flow. The HPLC column is the heart of HPLC system because the separation is occurred in this section. The detector is used to monitor an electronic signal of an eluted substance passing through the column. There are several detectors which can be coupled with HPLC. Generally, UV-Vis detector is the most common used. The detector monitors the absorption of UV or visible light of the substance at a specific wavelength. A display unit of most HPLC systems is a computer which integrates the response of the detector of each substance and presents in chromatogram. The characteristic peak of each substance is shown in a different retention time and peak area which are automatically calculated by the computer. The schematic diagram of HPLC is shown in Figure 5.

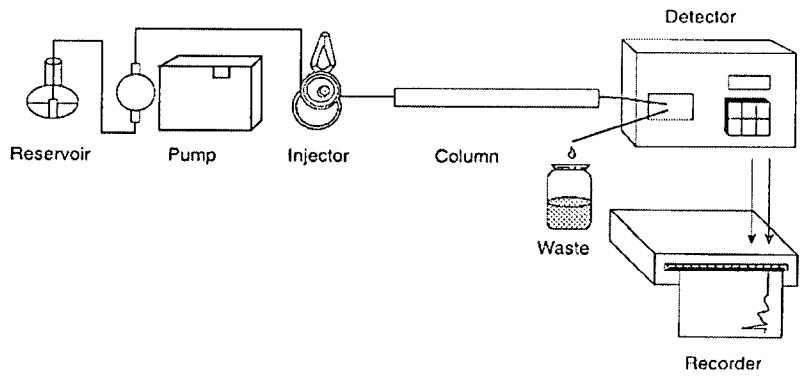


Figure 5 The schematic diagram of HPLC

Source: McMaster M.C., 2007

The basic terms and parameters

There are many basic terms and parameters used in HPLC analysis. These basic terms and parameters are shown in Table 7.

Table 7 The basic terms and parameters in HPLC analysis (Dong, 2006)

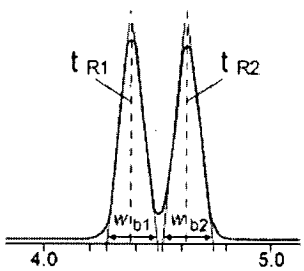
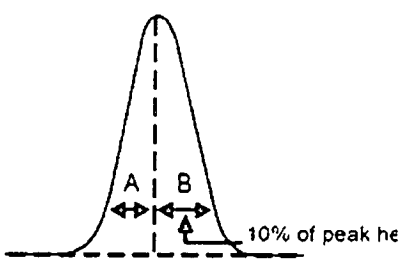
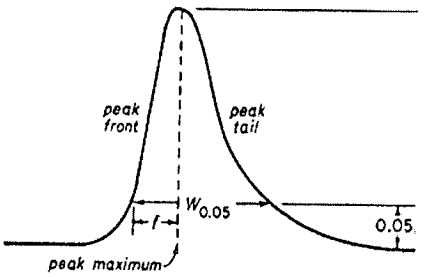
Parameter	Definition	Criterion
Resolution (R_s)	The measurement of degree of the separation of two adjacent analytical peaks Calculate equation; $R_s = (t_{R2}-t_{R1})/[(W_{b1}+W_{b2})/2]$  	$R_s = 0$; complete co-elution or not separation $R_s = 0.6$; a shoulder is discernible or slight partial separation $R_s = 1$; a partial separation and is the minimum separation required for quantitation $R_s = 1.5$; baseline separation
Asymmetry factor (A_s)	The measurement of the degree of peak symmetry Calculate equation; $A_s = B/A$  	$A_s = 1$; Asymmetry peak $A_s > 1$; Tailing peak $A_s < 1$; Fronting peak

Table 7 (Cont.)

Parameter	Definition	Criterion
Tailing factor (T_f)	The measurement of the degree of peak symmetry Calculate equation; $T_f = W_{0.05}/2f$	$T_f = 1$; Asymmetry peak $T_f > 1$; Tailing peak $T_f < 1$; Fronting peak



Sunscreen analysis by HPLC

As stated above, HPLC methods coupled with a UV detector is gained interest in this study because it is a simple, fast and economic method. In addition, HPLC is available in most of cosmetic companies. There are many HPLC methods that have been published for the determination of UV filters in sunscreen products.

Chisvert, et al. (2001a) determined seven UV filters simultaneously, using a C18 (125 x 4 mm, 5 μm) reverse phase column. The seven UV filters analyzed were 4-methylbenzylidene camphor, benzophenone-3, benzophenone-4, ethylhexyl dimethyl PABA, ethylhexyl salicylate, ethylhexyl methoxycinnamate and homosalate. An isocratic mobile phase consisted of ethanol: water: acetic acid (70: 29.5: 0.5, v/v/v) was used in the analysis. UV measurements were carried out at 313 nm. Acetic acid was used to decrease the peak tailing of benzophenone-3. The total analysis time was 25 min. Chrisvert, et al. (2001b) also modified the developed method by adding 65.4 mM of hydroxypropyl-β-cyclodextrin into the isocratic system of ethanol: water: acetic acid (70: 29.5: 0.5, v/v/v) to provide a good separation and a short retention time in the determination of seven UV filters. However, the total analysis time was only 5 min shorter than the previous system (i.e. 20 min) without hydroxypropyl-β-cyclodextrin as the mobile phase modifier.

Smyrniotakis and Archontaki (2004) developed HPLC system for the determination of four UV filters including ethylhexyl salicylate, ethylhexyl methoxycinnamate, methylene bis-benzotriazolyl tetramethylbutylphenol and octocrylene by C18 Column (250 x 4.6 mm, 5 μm). The composition of methanol: acetonitrile (90:10, v/v) was used as an isocratic mobile phase. UV absorption measurements were carried out at 313 nm. The total analysis time was 20 min. The major advantage of this system is the extremely hydrophobic compound methylene bis-benzotriazolyl tetramethylbutylphenol can be determined along with a hydrophilic ethylhexyl salicylate, ethylhexyl methoxycinnamate and octocrylene. However, the system can be simultaneously determined only four UV filters which might not covers all of currently used UV filters at the present.

Schakel, et al. (2004) determined sixteen UV filters including 4-aminobenzoic acid, benzophenone-3, benzophenone-4, butylmethoxy dibenzoylmethane, diethylhexyl butamidotriazone, drometrizoletrisiloxane, ethylhexyl dimethyl-aminobenzoate,

ethylhexyl methoxycinnamate, ethylhexyl salicylate, ethylhexyl triazone, homosalate, isoamyl-p-methoxycinnamate, methylbenzylidene camphor, octocrylene, phenylbenzimidazole sulphonic acid and terephthalidenedicamphor sulfonic acid. The HPLC condition composes of reverse phase C18 column (125 x 4.6 mm, 5 μm) and a gradient of ethanol-aqueous acetate buffer containing 0.2 mM of EDTA, with a flow rate of 1.0 mL/min, detected wavelength at 313 and 360 nm. It was observed that EDTA could be a chelating for inactivation metal proteins and provided more symmetric peak shape. However, the use of high concentration of EDTA could lead to a poor resolution of some peaks. Therefore, EDTA was added at the concentration of 0.2 mM into the mobile phase that could successfully elute sixteen UV filters within 32 min. However, the caution of this method is EDTA may contaminate with the pump. In addition, ethanol–acetate buffer used for diluting the PABA could result in the peak splitting. Therefore this method cannot be applied with the sunscreen product containing PABA.

Simeoni, et al. (2005) used various stationary phases such as cyanopropyl-bonded silica column, phenyl-bonded column and octadecyl-silica column to determine 4-methylbenzylidene camphor, benzophenone-3, butylmethoxy dibenzoylmethane, ethylhexyl dimethylamminobenzoate, ethylhexyl methoxycinnamate, ethylhexyl salicilate, phenylbenzimidazolesulphonic acid and octocrylene. The cyanopropyl-bonded silica column with the mobile phase containing methanol–acetonitrile–tetrahydrofuran–water (40:10:10:40, v/v/v/v) and 0.5% (v/v) acetic acid was suitable for the determination of eight UV filters in sunscreen products. UV–Vis detector was set at wavelength 320 nm. In addition, the method was applied to determine the extreme hydrophobic methylene bis-benzotriazolyl tetramethylbutylphenol, benzophenone-3, butylmethoxy dibenzoylmethane and ethylhexyl methoxycinnamate within 14.2 min.

Gaspar and Maia Campos (2006) evaluated the photostability of different UV filters combinations in a sunscreen formulation by using HPLC. A C18-column (250 x 4 mm, 5 μm ODS) was used with an isocratic mobile phase consisting of methanol and water (88:12, v/v). UV detection was carried out at 325 nm. The UV filters analyzed in this study were 4-methylbenzylidene camphor, benzophenone-3, butylmethoxy dibenzoylmethane, ethylhexyl salicylate, ethylhexyl methoxycinnamate and octocrylene. The total analysis time was 27 min.

De Orsi, et al. (2006) developed the gradient method for quantitative analysis of eleven UV filters including 4-methylbenzylidene camphor, benzophenone-3, benzophenone-4, bis-ethylhexyloxyphenol methoxyphenyltriazine, butylmethoxy dibenzoylmethane, diethylaminohydroxybenzoyl hexyl benzoate, ethylhexyl methoxycinnamate, ethylhexyl triazone, methylene bis-benzotriazolyl tetramethyl butylphenol, octocrylene and phenylbenzimidazole sulfonic acid. Three different methods were achieved for the quantification of UV filters by controlling the column temperature at 35°C and varying a stationary phase, mobile phase and flow rate. The researchers performed three methods. Method A, a C18-column (150 x 4.6 mm, 5 μm), a gradient of acetonitrile and water (adjusted to pH 3.0 with 1 M perchloric acid) with a flow rate of 1.0 mL/min were evaluated. Method B, a C8-column (150 x 4.6 mm, 5 μm), an isocratic of methanol and acetonitrile (1:1, v/v) with a flow rate 1.0 mL/min were employed. Method C, a RP-amide C16 column (250 x 4.6 mm, 5 μm), methanol and water (adjusted to pH 3.0 with 1 M perchloric acid) at the ratio 66:35, v/v, with a flow rate 2.0 mL/min were used. UV detectors were carried out at 280, 300, 310 or 360 nm. However, the developed method could not provide a simultaneous determination of all eleven UV filters in a single analysis.

Dencausse, et al. (2008) used a C18-column (150 x 4.6 mm, 5 μm) and the suitable wavelength at 330 nm for the analysis of four UV filters including benzophenone-3, butylmethoxy dibenzoylmethane, ethylhexyl methoxycinnamate and bis-ethylhexyloxyphenol methoxyphenyltriazine under ternary gradient mixture of tetrahydrofuran, acetonitrile and an aqueous solution of acetic acid. However, this method could detect only four UV filters within 30 min.

Imamavic, et al. (2009) developed HPLC system for the identification and determination of UV filters including benzophenone-3, butylmethoxy dibenzoylmethane and ethylhexyl methoxycinnamate by varying types of stationary phase and compositions of mobile phase. The separation of UV filters was achieved using C8 column and an isocratic elution mode. The mobile phase composes of acetonitrile and 0.5% phosphoric acid (70: 30, v/v). The column temperature was controlled at 42°C and UV measurements were carried out at 358 nm. The system, as mentioned above, can analyze only three hydrophilic UV filters within 16 min.

Scalia, et al. (2010) developed HPLC system for the determination of sunscreen photostability. The UV filters were benzophenone-3, butylmethoxy dibenzoylmethane and ethylhexyl methoxycinnamate. A SB-CN column (150 x 4.6 mm, 5 μm,Zorbax®) and the mobile phase consisted of methanol, acetonitrile, tetrahydrofuran, water (45: 15: 10: 30, v/v/v/v) and 0.5% (v/v) acetic acid were examined. The UV detector was carried out at 330 nm. However, only three UV filters were detected within 6 min.

Nyeborg, et al. (2010) validated the simultaneous method under gradient elution. The method was performed on a C18-column (150 x 4.6 mm, 5 μm). Detected wavelength in the analysis was 312 nm. The analyzed UV filters were benzophenone-3, bis-ethylhexyloxyphenol methoxyphenyltriazine, butylmethoxy dibenzoylmethane, diethylaminohydroxybenzoyl hexyl benzoate, diethylhexylbutamidotriazone, ethylhexylmethoxycinnamate, ethylhexyl salicylate, ethylhexyltriazone, isoamyl*p*-methoxycinnamate, methylene bis-benzotriazolyl tetramethylbutylphenol, octocrylene and phenylbenzimidazole sulfonic acid. However, the sensitivity of butylmethoxy dibenzoylmethane is quite low, so the authors suggested that UV detection at 358 nm is more preferable than at 312 nm for the compound. Unfortunately, this method was not able to determine all twelve filters simultaneously. This is due to some formulations contain butylmethoxy dibenzoylmethane in a low concentration and thus the analysis must be performed in both wavelengths.

Gaspar and Maia Campos (2010) developed a gradient HPLC method to evaluate the photostability of UV filters in sunscreen formulations containing vitamin A and E. The UV filters were benzophenone-3, ethylhexyl methoxycinnamate and octocrylene. The separation was achieved by using C18 column (250 x 4 mm), flow rate at 1.0 mL/min, detection wavelength at 325 and 235 nm. The composition of methanol, isopropanol and water were used as the mobile phase. Three UV filters were successfully determined within 27 min.

Liu and Wu (2011) presented the HPLC method for the simultaneous analysis of eleven UV filters including 4-aminobenzoic acid, 4-methylbenzylidene camphor, octocrylene, benzophenone-3, benzophenone-4, ethylhexyl methoxycinnamate, ethylhexyl salicylate, homosalate, isoamyl-p-methoxycinnamate and methylene bis-benzotriazolyl tetramethylbutyl phenol. The analysis was achieved employing methanol, tetrahydrofuran and aqueous perchloric acid as the mobile phase under the

gradient elution mode. A C18-column (250 x 4.6 mm, 5 μm) and the detection wavelength at 311 nm were used to analyze UV filters. Total analysis time was around 20 min. However, the use of high concentration of tetrahydrofuran and aqueous acid solution in the mobile phase can cause a column degeneration which can lead to a change in selectivity, peak shape and peak resolution.

Wharton, et al. (2011) determined seven UV filters including 4-methylbenzildene camphor, benzophenone-3, butylmethoxy dibenzoylmethane, ethylhexyl dimethyl PABA, ethylhexyl methoxycinnamate, ethylhexyl salicylate and octocrylene. The mobile phase containing ethanol and 1% acetic acid was use in the gradient elution mode in order to analyze sunscreen and others cosmetic products. The C18 BDS-column (100 x 4.6 mm, 5 μm), the flow rate at 1.0 mL/min and the wavelength at 313 nm were used to separate UV filters. Only hydrophilic UV filters could be separated with the total analysis time of 7 min.

Peruchi and Rath (2012) developed an isocratic HPLC system for the assay of eight sunscreen agents in suncare products by using C18 column (250 x 4 mm, 5 μm). The eight UV filters were 4-methylbenzylidene camphor, benzophenone-3, butylmethoxy dibenzoylmethane, ethylhexyl dimethyl PABA, ethylhexyl salicylate, ethylhexyl methoxycinnamate, homosalate and octocrylene. The UV detector was carried out at 238 nm. In this study, the column temperature was observed between 20-50°C. The results showed that column temperature strongly influences the retention time and the resolution of compounds. The higher temperature above 30°C results in a poor resolution while a co-elution was observed at 45°C. The mixture of methanol: water (88:12, v/v) was used as a mobile phase. However, the separation was achieved within 16 min when the column temperature was controlled at 20 °C.

In conclusion, the published HPLC methods for the determination of UV filters in sunscreen products are summarized in Table 8. However, as mentioned above that the trends of UV filters commercially used in the sunscreen products have been changed overtime. Some new filters are introduced into the market and there is no the up to date published HPLC method covers all of currently used UV filter in Thailand.

Table 8 The summary of published HPLC methods for the determination of UV filters in sunscreen products

Authors	HPLC conditions	Analyzed UV filters
Chisvert, et al. (2001a)	C18 (125 x 4 mm, 5 μm), isocratic of ethanol: water: acetic acid (70 : 29.5 : 0.5, v/v/v), UV 313 nm	4-MBC, BZ-3, BZ-4, ED-PABA, EHMC, EHS and HMS
Chisvert, et al. (2001b)	C18 (125 x 4 mm, 5 μm), isocratic of ethanol: water: acetic acid (70 : 29.5 : 0.5, v/v/v) containing 65.4 mM of hydroxyl-propyl-β-cyclodextrin,UV 313 nm	BMDBM, BZ-3, BZ-4, ED-PABA, EHMC, EHS and HMS
Smyrniotakis and Archontaki (2004)	C18 (250 x 4.6 mm, 5 μm), isocratic of methanol: acetonitrile (90:10, v/v), UV 313 nm	EHMC, EHS MBBT and OCR
Schakel, et al. (2004)	C18 (125 x 4.6 mm, 5 μm), gradient of ethanol: aqueous acetate buffer containing 0.2 mM of EDTA, UV 313 and 360 nm	4-MBC, BMDBM, BZ-3, BZ-4, DBT, DTS, ED-PABA, EHMC, EHS, EHT, HMS, IMC, OCR, PABA, PBSA and TDSA
Simeoni, et al. (2005)	cyanopropyl-bonded silica column, gradient of methanol, acetonitrile, tetrahydrofuran and aqueous acetic acid, UV 320 nm	4-MBC, BMDBM, BZ-3, ED-PABA, EHMC, EHS, PBSA and OCR

Table 8 (Cont.)

Authors	HPLC conditions	Analyzed UV filters
Gaspar and Maia Campos (2006)	C18 (250 x 4.6 mm, 5 µm), isocratic of methanol: water (88:12, v/v), UV 325 nm	4-MBC, BMDBM, BZ-3, EHMC, EHS and OCR
De Orsi, et al. (2006)	Three different methods: *Method A*; C18 (150 x 4.6 mm, 5 µm), a gradient of acetonitrile and water (adjusted at pH 3.0 with 1 M perchloric acid), *Method B*; C8 (150 x 4.6 mm, 5 µm), isocratic of methanol: acetonitrile (1:1, v/v), *Method C*; RP-amide C16 (250 x 4.6 mm, 5 µm), isocratic of methanol: water, 66:35, v/v (adjusted at pH 3.0 with 1 M perchloric acid), UV 280, 300, 310 or 360 nm	4-MBC, BEMT, BZ-3, BZ-4, BMDBM, ED-PABA, EHMC, EHT, MBBT, OCR and PBSA.
Dencausse, et al. (2008)	C18 (150 x 4.6 mm, 5 µm) gradient of tetrahydrofuran, acetonitrile and an aqueous solution of acetic acid, UV 330 nm	BEMT, BMDBM, BZ-3 and EHMC
Imamavic, et al. (2009)	C8, isocratic of acetonitrile: 0.5% phosphoric acid (70: 30, v/v), UV 358 nm	BMDBM, BZ-3 and EHMC

Table 8 (Cont.)

Authors	HPLC conditions	Analyzed UV filters
Scalia, et al. (2010)	SB-CN (150 x 4.6 mm, 5 μm), isocratic of methanol: acetonitrile: tetrahydrofuran: water (45: 15: 10: 30, v/v/v/v) containing 0.5% (v/v) acetic acid, UV 330 nm	BMDBM, BZ-3 and EHMC
Nyeborg, et al. (2010)	C18 (150 x 4.6 mm, 5 μm), gradient of ethanol and water acidified with 1% of 0.1 mol L^{-1} H_3PO_4, UV 312 nm	BEMT, BMDBM, BZ-3, DBT, ED-PABA, EHMC, EHS, EHT, IMC, MBBT, OCR and PBSA
Gaspar and Maia Campos (2010)	C18 (250 x 4 mm, 5 μm), gradient of methanol, isopropanol and water, UV 325 and 235 nm	BZ-3, EHMC and OCR
Liu and Wu (2011)	C18 (250 x 4.6 mm, 5 μm), gradient of methanol, tetrahydrofuran and perchloric acid aqueous solution, UV 311 nm	4-MBC, BZ-3, BZ-4, EHMC, EHS, HMS, IMC, MBBT, OCR and PABA
Wharton, et al. (2011)	C18 (100 x 4.6 mm, 5 μm), gradient of ethanol and 1% acetic acid, UV 313 nm	4-MBC, BMDBM, BZ-3, ED-PABA, EHMC, EHS, and OCR
Peruchi and Rath (2012)	C18 (250 x 4 mm, 5 μm), isocratic of methanol: water (88:12, v/v), UV 238 nm	4-MBC, BMDBM, BZ-3, ED-PABA, EHS, EHMC, HMS and OCR

Where: PABA; 4-aminobenzoic acid, 4-MBC; 4-methylbenzylidene camphor, BZ-3; benzophenone-3, BZ-4; benzophenone-4, BEMT; bis-ethylhexyloxyphenol methoxyphenyltriazine, BMDBM; butylmethoxy dibenzoylmethane, DBT;

diethylhexylbutamidotriazone, DTS; drometrizoletrisiloxane, ED-PABA; ethylhexyl dimethyl PABA, IMC; isoamyl-p-methoxycinnamate, EHS; ethylhexyl salicylate, EHMC; ethylhexylmethoxycinnamate, HMS; homosalate, MBBT; methylene bis-benzotriazolyl tetramethylbutylphenol, OCR; octocrylene, PBSA; phenylbenzimidazolesulphonic acid, TDSA; terephthalidenedicamphor sulfonic acid