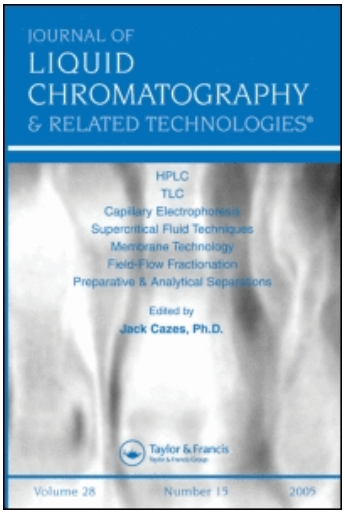

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THE CHEMISTRY AND HPLC ANALYSIS OF CHEMICAL SUNSCREEN FILTERS IN SUNSCREENS AND COSMETICS

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THE CHEMISTRY AND HPLC ANALYSIS OF CHEMICAL SUNSCREEN FILTERS IN SUNSCREENS AND COSMETICS

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INTRODUCTION

Outdoor activities are enjoyed by many people. However, exposure to sunlight can have good and bad effects on the skin and health. While sunlight helps with the formation of vitamin D, it also contributes towards premature aging and skin cancer. Recently, the public has become more aware of these effects. Therefore, the development of effective sunscreens is very important since they allow people to spend a longer time in the sun while reducing the adverse effects.(1,2)

The optical region of the solar spectrum consists of ultraviolet (UV), visible, and infrared (IR) radiation. The UV region has the highest energy and is responsible for the damage caused to skin and hair.(3) The UV region is divided into UVA (320-400nm), UVB (290-320nm), and UVC (200-290) nm. UVC, with the highest energy, is the most dangerous but is absorbed before it reaches the earth.(2,4)

The wavelength of the light incident on the skin affects the depth to which the radiation penetrates and how much it penetrates. Between 300 to 350 nm only about 20% passes the epidermis (outer layer of the skin), increasing to 80% at 550 nm(5) (Fig. 1). UVB light contributes towards sunburn and has been

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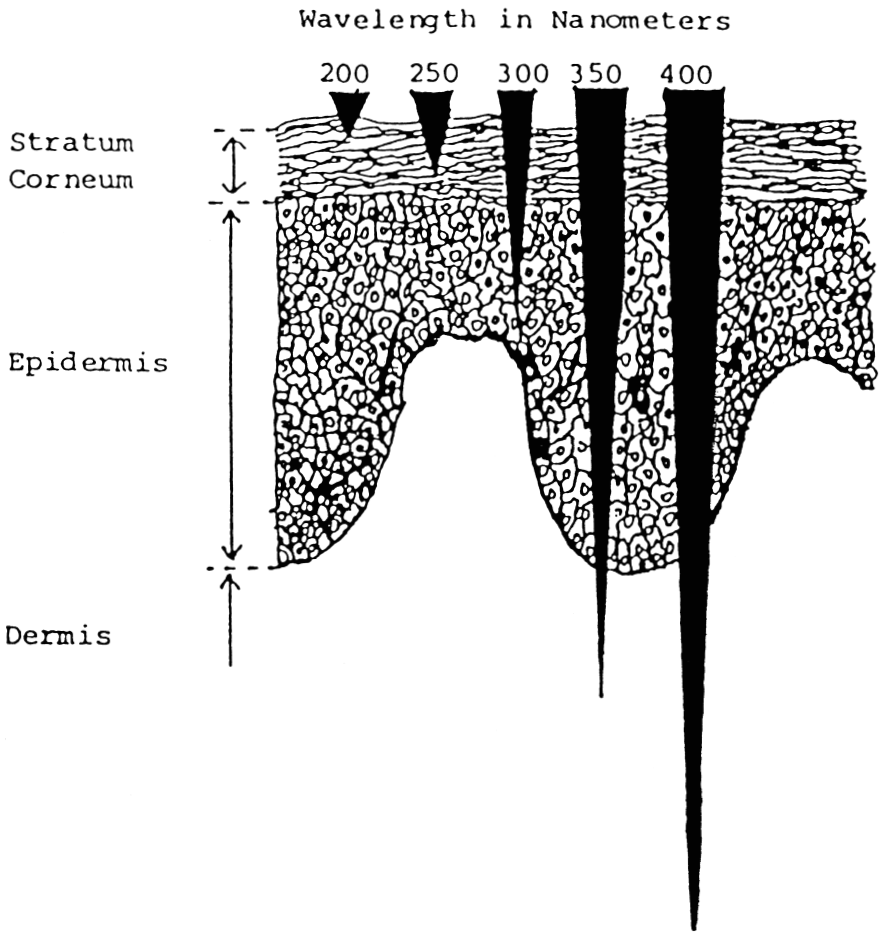


Figure 1. Schematic representation of light penetration into the skin (reprinted with permission from Ref. 3).

linked to skin cancer and suppression of the immune system. UVA has the lowest energy and penetrates the deepest into the skin.(2,4) It is linked to premature aging such as loss of collagen, change in connective tissue, and decrease in the number of blood vessels.(2,4,6). There is some difference in the literature as to whether the UVB region causes tanning(4) or whether it is the UVA.(3,7)

Sunscreens are applied to the skin to protect the skin from ultraviolet radiation. They contain UV filters of a physical or chemical nature.(8) The physical blockers can reflect the full optical spectrum, whereas the chemical sunscreens

have a specific range in which they absorb.(9) Sunscreens are rated by a sun protection factor (SPF) that denotes their effectiveness for protection from sunburn.(8) The SPF indicates the length of time an individual can stay in the sun without damage to the skin. Without risking sunburn, individuals can stay in the sun with SPF 15, fifteen times as long as if they were not wearing sunscreen. Since sunburn is attributed to UVB radiation, the SPF ratings only apply to this type of radiation.(4)

UV absorbers are classified according to the area of the UV spectrum; for example, benzophenones, anthranilates, and dibenzoylmethanes are labeled UVA absorbers. Since benzophenones and anthranilates absorb from 300-350 nm they are considered broad-spectrum sunscreens. The UVB region is protected by para-aminobenzoic acid (PABA) and its derivatives, as well as, by salicylates, camphor derivatives, and the most widely used filters, cinnamates.(3,9,10)

The first commercially available chemical sunscreen agent was PABA, which is water-soluble. Derivatives were developed that were soluble in oil and, hence, were widely used in sunscreen formulations.(2) Investigation into alternative sunscreen agents began when it was found that PABA and its derivatives caused allergic reactions in a small section of the population.(2,11) By the end of the 1980's many products had been developed, that had very high SPF's but did not contain PABA.(12) In order to achieve a high SPF, manufacturers can increase the concentrations of the filter so they absorb more. However, there are maximum allowed concentrations; beyond this concentration the sunscreen agents irritate the skin. Another option is to use combinations of chemical filters to achieve this goal.(2) Combinations work synergistically, protect better, and are more economical than one sunscreen filter. During the 90's quite a few sunscreens were developed having four or more filters. One of the SPF 15 UV lotions being marketed today (Lubriderm daily UV lotion SPF 15) contains octyl methoxycinnamate 7%, octyl salicylate 4%, and oxybenzone 3%.(13)

The combinations enabled protection from a wider area of the spectrum. It is believed that these developments have made sunscreens safer, more efficient, and more cost effective.(13,14,15) However, there were concerns raised at the 55th annual meeting of the society of toxicology. It was noted that category I agents (considered safe and effective) are used in combination with other category I filters without reference to the medium. The influence of the medium on active ingredients in combination sunscreens needed to be studied, since photodecomposition and the extent of biological reactions are related to the medium. It was noted, that long-term toxicity information on the combination sunscreens was of further interest.(16)

The inactive ingredients that can affect the effectiveness of the sunscreen are materials such as water, emulsifiers, oils, gums, and polymers. The amounts of these materials vary with the brand.(17) Sunscreen agents are also used in

moisturizers, makeup, lipstick, shampoos, hair gels, and hair mousses, in order to prevent the degradation of cosmetic products by sunlight.(10,18)

THE CHEMISTRY OF SUNSCREENS

Photochemistry of Sunscreens

Chemical sunscreen filters are organic molecules with a high degree of unsaturation; thus, a part of the molecule is a UV absorbing chromophore. They work by absorbing photons and promoting electrons from a D_0 ground state to an excited state ^1D within the molecule.(2,5,19)

$$D_0 + h\lambda \rightarrow {}^1D \quad (5)$$

The excited molecule returns to ground state in several ways. Usually, energy is transferred to a different state or molecule through internal conversion and then the molecule returns to ground state by vibrational relaxation or fluorescence (radiates a longer wavelength than the incident radiation). The excited singlet molecule, ^1D, can also be converted to the lower energy triplet state ^3D via intersystem crossing and return to the ground state through phosphorescence or vibrational relaxation. This cycle is repeated, effectively shielding the skin from the UV radiation (Fig. 2).(2, 5,7)

The excited states also undergo photochemical reactions. Activated compounds, depending on the nature of the compound and solvent, sometimes decompose into non-UV absorbing species.(5) Other reactions include the formation of free radicals or singlet oxygen.

The singlet state has a short lifetime 10^{-9} to 10^{-8} that limits the number of reactions taking place from this state. With a lifetime of 10^{-4} or greater the more important reactions take place from the triplet state. Several factors govern the reactions from the triplet state: energy and lifetime of the state, concentration and identity of other reactants, and the rates and activation energies of competing reactions.(7)

One of the most important photoreactions from the triplet state involve ground state oxygen receiving energy from the excited molecule and forming singlet oxygen.

$$^3D + {}^3O_2 \rightarrow {}^1O_2 + D_0 \quad (5)$$

Singlet oxygen reacts with several molecules found in the skin as well as topically applied products, either destroying another molecule when it is quenched or turning another molecule into a free radical. In this manner, even

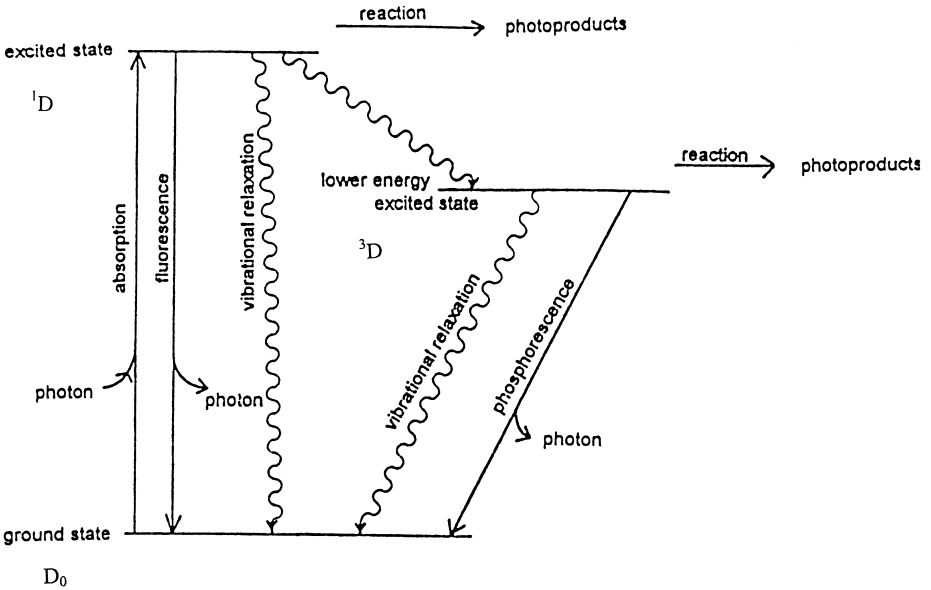


Figure 2. Diagram showing absorption of photon and release of energy from excited molecule (adapted with permission from Ref. 2).

molecules that do not absorb UV radiation can react and the results can be extremely damaging to the skin.(5) For example, UVA reacts with trans-urocanic acid, a molecule in the skin, to form singlet oxygen, which causes photodegradation of the skin and results in symptoms of aging. Some scientists feel that the formation of the singlet oxygen from trans-urocanic acid is also a factor in some types of cancer.(6)

Properties of Sunscreens

Sunscreen absorbance is dependent on the structure and symmetry of the molecule. Delocalization, electron releasing groups, the acidity/alkalinity and polarity of the compounds are all related factors. Solvent properties, such as pH and polarity that relate to the ability of the sunscreen to solvate, affect compounds in different manner.(3)

Conditions that support increased delocalization, for example, acidic compounds in alkaline conditions forming anions, result in a lowering of the energy required to promote an electron and causes a higher λ_{max} to be observed

(bathochromic shift). Effective delocalization also results in a higher extinction coefficient. A hypsochromic shift (lower λ_{max}) is observed with the protonation of an alkaline compound in an acidic medium, since delocalization is reduced.(3)

The polarity of the solvent causes either stabilization of the ground state or excited state through increased solvation. The ground state of a polar sunscreen is stabilized in a polar solvent, resulting in increased transition energy and a hypsochromic shift. In a sunscreen agent where the excited state is more polar than the ground state, the excited state is stabilized in a polar solvent. This stabilization lowers the transition energy resulting in a bathochromic shift (Fig. 3).(3)

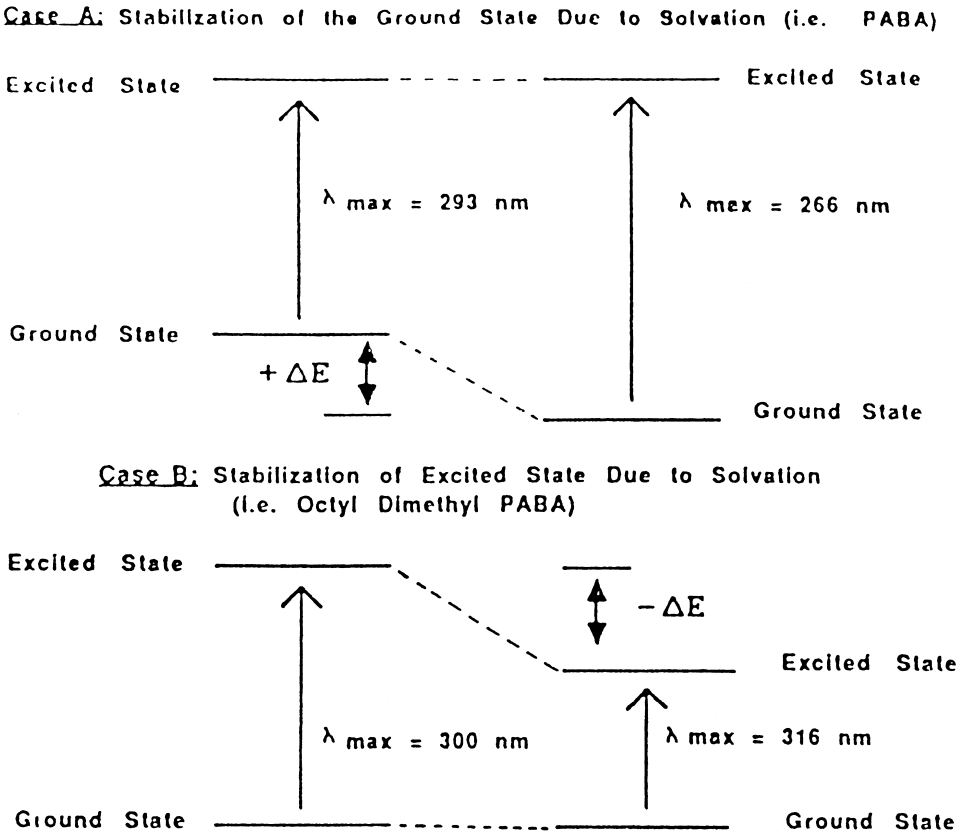


Figure 3. Energy diagram depicting the stabilization of the ground state and the excited state (reprinted with permission from Ref 3).

p-Amino Benzoic Acid (PABA) and Its Derivatives

PABA has a carboxylic acid on one end and an amine group on the other end that are both sensitive to pH changes (Fig. 4). PABA undergoes delocalization that allows moderate absorbance in the UVB region. However, the λ_{max} is at the low end of the spectrum and this decreases the effectiveness.(3,20) The carboxylic and amine groups cause the molecule to be extremely polar and when PABA is placed in a polar solvent, λ_{max} is lowered even more. In the polar solvent, the molecule also undergoes intermolecular bonding that causes crystallization affecting the consistency of the formulation.(3,20)

PABA derivatives tend to have good stability. They can be derivatized without causing great changes in their absorption spectrum.(21) Subsequent derivatives were created protecting the two groups; one of the most effective derivatives is Padimate-O, which has an extremely high extinction coefficient (especially in polar solvents) and is easier to use since it is not crystalline. Although Padimate-O is still affected by the solvent, the λ_{max} remains within the UVB range.(3,20)

Salicylates

The UVB absorbers, salicylates, are ortho-substituted compounds that undergo internal hydrogen bonding (Fig. 5). Electrons in the conjugated carbonyl group are loosened, decreasing transition energy and exhibiting a λ_{max} of about 300 nm, higher than the corresponding para- and meta- compounds (Fig. 6). This phenomenon is called the ortho effect. Additionally, the hydrogen bonding makes the hydroxyl and carbonyl group unavailable for interaction with the

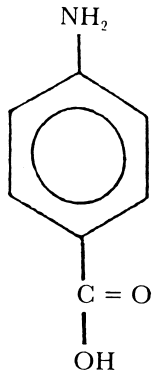


Figure 4. Structure of para-aminobenzoic acid.

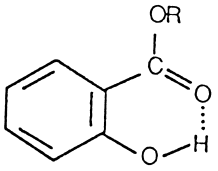


Figure 5. Structure of salicylates showing internal hydrogen bonding (reprinted with permission from reference 20).

solvent making salicylates relatively stable. However, the ortho position results in a steric strain that changes the symmetry of the molecule and decreases the molar extinction coefficient making salicylates weak absorbers.(3,20) Salicylates can show great differences in their spectral stabilities. For example homo menthylsalicylate has been known to show excellent stability in ethanol/propanediol while phenyl salicylate shows poor stability.(21)

Cinnamates

Cinnamate molecules have extra unsaturation that results in greater delocalization (Fig. 7). Cinnamates have λ_{max} of about 305 nm (in the UVB region) and a high extinction coefficient. They also absorb well in the UVA region and are oil soluble making them a popular choice in sunscreen formulations(2,3,20) One of the most widely used sunscreen absorbers is trans-2-ethylhexyl-p-methoxycinnamate (EHMC) (Fig. 8). The electron releasing methoxy group causes a high molar absorptivity, but on exposure to light the absorbance of trans–EHMC is decreased.(19) Broadbent et al(19) showed that, under UV radiation an equilibrium between the cis and trans isomers occurs, and this eventually reaches a photo-stationary state. The cis isomer was present in greater quantity at the stationary state and this isomer is a less efficient UV absorber. Under the conditions used, Broadbent et al found no significant other photoproducts.

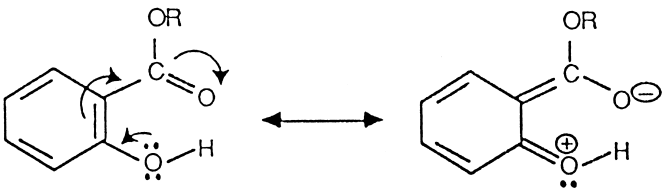


Figure 6. Internal resonance delocalization of salicylates (reprinted with permission from reference 20).

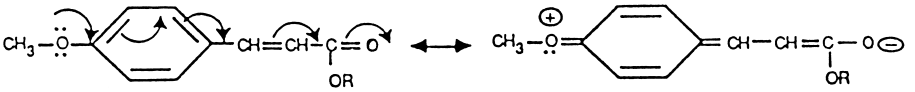


Figure 7. Internal resonance delocalization of cinnamates (reprinted with permission from reference 20).

Camphor Derivatives

Camphors are more widely used outside of the US (Fig. 9). They protect the UVB in the 290-300 nm region and have high molar absorptivity coefficients.(20) Benzylidene camphor derivatives tend to be relatively stable regardless of medium; however, like cinnamates, they isomerize reaching a photo-stationary equilibrium quickly after exposure to light. The irreversible disappearance of the sunscreen on exposure to light tends to be low.(23)

Dibenzoylmethanes

Dibenzoylmethanes are highly conjugated and, therefore, have a high molar extinction coefficient. They undergo keto-enol tautomerism (Fig. 10) with the λ_{max} of the enol in the UVA region (about 350nm) and the keto in the UVC region. The hydroxyl group in the dibenzoylmethane structure has an ortho position and this stabilizes the enol so dibenzoylmethanes are classified as UVA absorbers.(3,20) Dibenzoylmethane derivatives are relatively unstable in non-polar solvents. They tend to fragment between the methylene group and an adjacent carbonyl group.(5)

Deflandre and Lang(23) indicate a difference in photostability of non-hydroxylated and hydroxylated compounds dibenzoylmethanes. In their experiments on the compounds that have a hydroxyl group ortho to the carbonyl group,

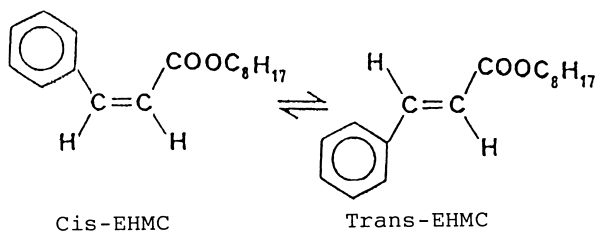


Figure 8. Cis-trans isomerism of octyl methoxy cinnamate (reprinted with permission from reference 22).

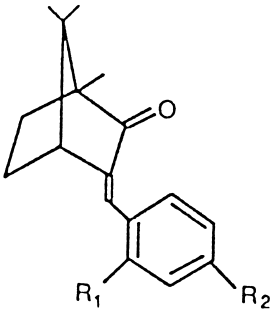


Figure 9. Structure of camphor derivatives.

the change in absorbance decreases much more rapidly on exposure to light than the non-hydroxylated compounds. Deflandre and Lang note that since dibenzoylmethanes photochemically react differently in varying formulations, that these results may have been different using other formulations.

One commonly used dibenzoylmethane is butyl methoxyl-dibenzoylmethane, which is known to be unstable to UV radiation. It yields products such as tert-butyl benzoic acid, tert-butyl benzene, and p-methoxy benzoic acid. Even

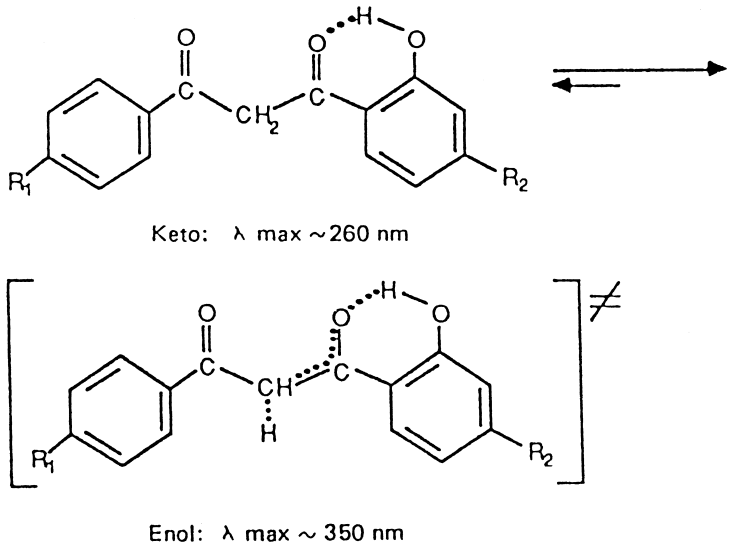


Figure 10. Keto-enol tautomerism of dibenzoylmethanes (reprinted with permission from reference 20).

in cosmetic formulations, it must be paired with other UV filters that improve the stability. With butyl methoxy dibenzoylmethane, reactions that cause loss of sunscreening ability are avoided by transferring energy to the other UV filters in the formulation.(24)

Benzophenones

Benzophenones are aromatic ketones and resonate more easily than the other UV absorbers since they are esters (Fig. 11) and, thus, have a high λ_{max}, in the UVA region. However, they are difficult to solubilize and have a tendency to cause allergic reactions. Benzophenones are greatly affected by the polarity of the solvent. Hypsochromic shifts reduce λ_{max} of polar benzophenones, to the very low end of the UVA, decreasing their effectiveness. In a polar solvent, dioxybenzone has a λ_{max} of 326 nm, with λ_{max} of 352 nm in a nonpolar solvent.(3,20)

It is often difficult to find a sunscreen agent that absorbs across most of the UVA region and in adequate magnitude to attenuate 90% or more of the incident radiation. Avobenzone meets this criterium and is also a significant UVB absorber.(7) However, it has photostability problems that tend to be dependent on the vehicle.(5) In general, benzophenones are similar to PABA derivatives in that they have good stability and are capable of derivatization without major spectral changes.(21)

Anthranilates

The UVA absorbers, anthranilates, are similar to salicylates in that they are affected by steric crowding due to the ortho position, have low extinction coefficients, and are not subject to solvent effects due to their internal hydrogen bonding. Anthranilates also undergo delocalization easily; this results in a smaller transition energy and a λ_{max} in the UVA region. Menthylanthranilate, one of the

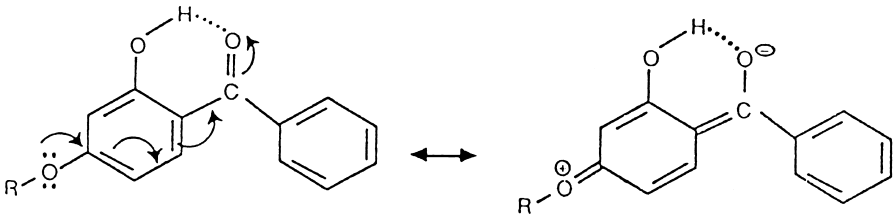


Figure 11. Internal resonance delocalization of cinnamates (reprinted with permission from reference 20).

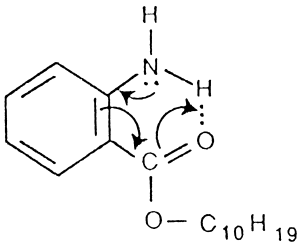


Figure 12. Electron delocalization of menthylanthranilate λ_{max}336 nm (reprinted with permission from reference 20).

few anthranilates approved for commercial use, has a λ_{max} of 336 nm. (Fig. 12)(3,20)

ANALYSIS OF SUNSCREENS

Developments in HPLC Analsis of Sunscreens

With the large numbers of sunscreens that have been developed and the complexity of the formulations, reliable ways of detecting concentrations of the sunscreen agents have become important. The sunscreen industry needs to check their products regularly for purity and for its spectroscopic characteristics. In several countries such as USA, Australia, and Japan there are regulations governing the UV absorbers' maximum allowed concentrations that can be included in formulations. Analyses are used in the development of new filters and formulations, to check stability and properties of filters and to analyze competitors' products.(25) As a part of quality control the contaminants and breakdown, products must be monitored since they may have toxic or allergic effects.

Analysis for Quality Control

Early work on the HPLC analysis of sunscreen agents in sun-creams was published by Masse et al.(26) in 1982. Major compounds were separated by reversed phase HPLC (RPLC) with isocratic elution. In 1983 Konig and Ryschka(27) analyzed the sunscreen agents in cosmetic products using both normal phase (NP-HPLC) and RPLC. They analyzed water-free and emulsified suntan formulations using normal phase HPLC; and water-soluble UV filters were separated by reversed phase HPLC.

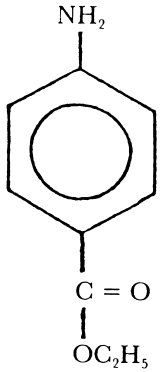


Figure 13. Structure of benzocaine.

In subsequent years, compliance with the regulations set out by the Council of the European Community (ECC) became the basis for several studies.(28,29,30,31,32). The ECC directive 76/768 indicated the sunscreen agents provisionally authorized for use and the allowed concentrations.(28,29,30) In addition, this directive forbid the presence of benzocaine, a contaminant in sunscreen preparations (Fig. 13). In 1984 Bruze et al.(33) separated PABA and benzocaine from PABA esters by TLC and HPLC (Fig. 14). This analysis is important since PABA and benzocaine are both known to cause sensitivity and allergies, or dermatitis for individuals. Although, benzocaine is not intentionally included in sunscreens it may be used in the manufacture of the UV filter and, hence, appears as a contaminant. Bruze et al. were able to measure, quantitatively, the presence of the two contaminants in samples of the four PABA esters. Subsequently, Gagliardi et al.(28,29,30) developed a RPPLC method that allowed simultaneous determination of sunscreen agents and the quantitative analysis of benzocaine. Gagliardi et al recommended their method for routine inspection of cosmetics to check conformance with EEC regulations.

Other papers mention the need for development of routine methods of analysis for the purpose of quality control, since some ingredients are known to cause allergy and irritation.(10,18,34,35,36,37)

Analysis for Photostability, Isomerism, and Degradation

In the analysis of sunscreens, another important area of research is the photostability and photoreactions of the sunscreen filters. These studies are necessary to know which sunscreens breakdown and need to be stabilized, the effects of the type of medium on the stability of the sunscreen and the shelf lives, and the

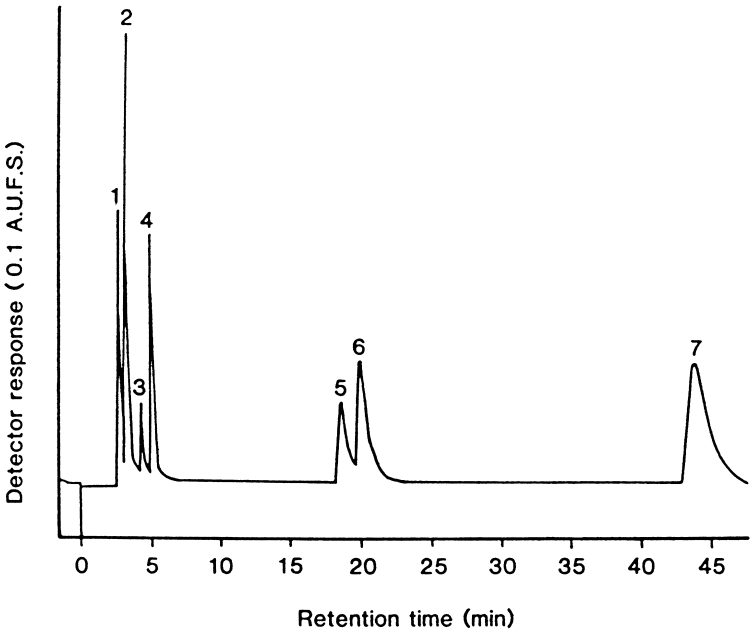


Figure 14. HPLC chromatogram of a mixture of para-aminobenzoic acid, benzocaine, glyceryl para-aminobenzoate from Nipa (0.005%, w/v, of each), 0.02%(w/v) amyl para-dimethylaminobenzoate and 0.03%(w/v) 2-ethylhexyl para-dimethylaminobenzoate. Mobile phase:acetonitrile/0.015 mol x 1^{-1} phosphoric acid 55/45 (v/v). Peaks : 1=glyceryl PABA; 2=PABA peak; 3=impurity in glyceryl PABA; 4=benzocaine; 5,6=amyl para-dimethylaminobenzoate, 7=2-ethylhexyl para-dimethylaminobenzoate. (adapted with permission from Ref. 33).

conditions during storage. In addition, it is possible that the photoproducts could be the cause of photoallergy or phototoxicity, and this is important in the choice of active ingredients and type of formulation chosen.(21)

Both the solvent and exposure to UV light contributes to the photochemical and degradation reactions. It is necessary to be aware of the products formed and how they affect the action of the sunscreen. Several HPLC studies have been done on the photostability of sunscreen products.(1,38,39,40,41) Meijer and Loden(1) used HPLC for the stability of 3 UV filters in a sun lotion; benzophenone-3, butyl methoxy dibenzoylmethane, and octyl methoxycinnamate (Fig. 15). They found octyl methoxy cinnamate likely to undergo isomerism and that the isomeric peak was increased in the presence of light. This was attributed to the cis/trans isomerism of octyl methoxycinnamate (shown in Fig. 8). The study indicated adequate sunscreening properties during storage for 2-3 years at room

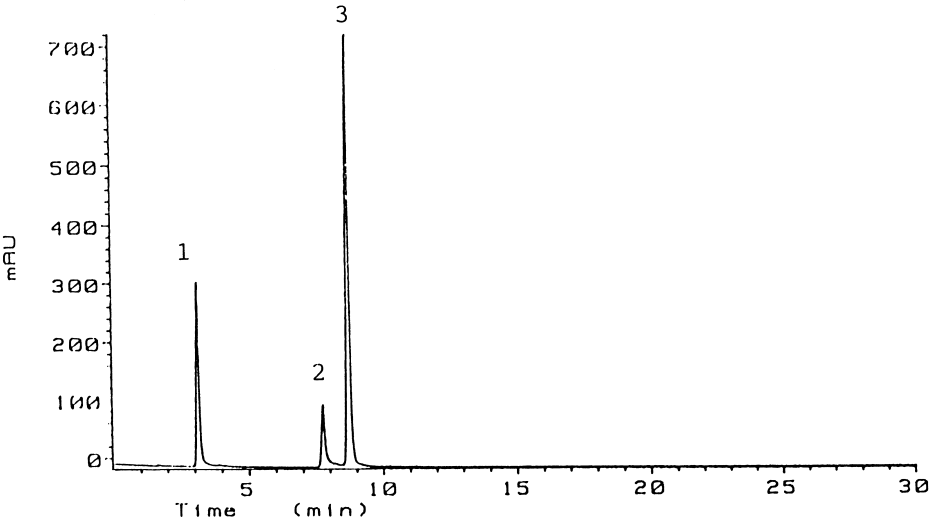


Figure 15. Chromatogram of a mixture of UV-filters equivalent to 1µg of each compound. 1. benzophenone, 2. butylmethoxydibenzoylmethane, 3. octylmethoxycinnamate. Detection at 325nm; 5µm C_8 column; gradient elution from 80% methanol to 100% methanol during 10 minutes – other phase 1% acetic acid in water; flow rate 1.0 mL/min; column temperature 25°C (adapted with permission from Ref. 1).

temperature. Shelf lives of sunscreen products should be known, as a decrease in effectiveness can be accompanied by an increase in degradation products. Therefore, Vanquerp et al.(38) used RPLC to study the photostability of sunscreen agents, and was able to calculate half-lives and the 90% shelf lives of the sunscreen agents.

Another important study involved the analysis of urocanic acid (UCA) isomers by De Orsi et al.(40) using HPLC. Trans-UCA is used in cosmetic formulations as an UV filter and is susceptible to conversion to the cis isomer, which facilitates immunosuppressive activity (Fig. 16).

In 1996 Berset et al.(41) proposed a protocol to determine the photostability of cosmetic UV filters. This in vitro model assessed the effectiveness of the filters after UV exposure and was applicable to UVB filters, with a slight modification for UVA filters. Results from UV spectroscopy were affected by isomerization, as the isomers had different extinction coefficients. The HPLC results reflected the total concentration of both isomers. This demonstrated the necessity for a spectroscopic result to be checked using a separation technique such as HPLC when measuring photostability.

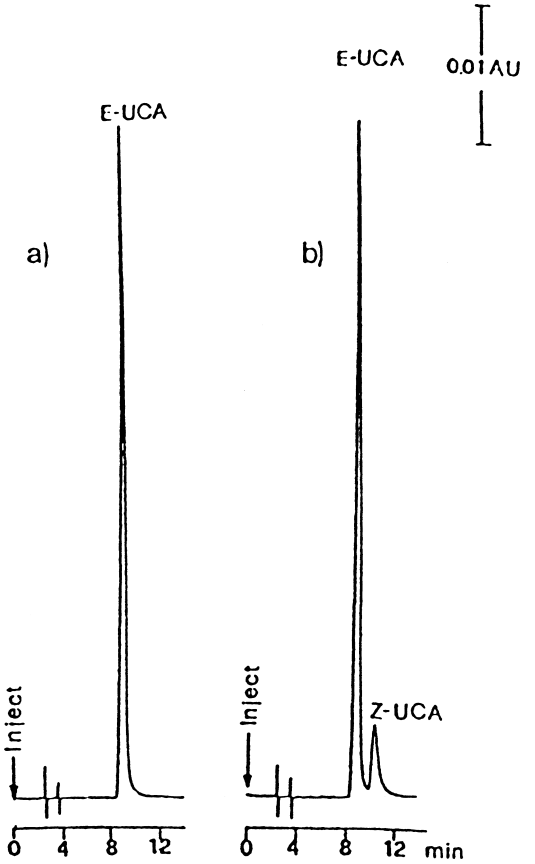


Figure 16. Typical chromatogram obtained at 263 nm for a) solution containing 10 μg mL^{-1} of E-UCA; b) the same solution after UV radiation. Mobile phase, acetonitrile-water containing 0.1M sodium perchlorate, pH 3 adjusted with 70% perchloric acid (2:98, v/v); flow-rate, 1.0 $mLmin^{-1}$; injection volume 10 μL; column temperature 25°C, detection wavelength (Varian 9050), 263 nm. The range of wavelengths analyzed by means of the photodiode array detector was 190-367 nm (adapted with permission for Ref. 40).

The degradation reactions make it difficult for chemists to formulate sunscreens with high SPFs. Sunscreens with high SPFs absorb significantly in the 290-340 nm range, so if the degradation reaction results in a shift to a wavelength outside this region, there will be a reduction in the SPF.(22) Since most of the sunscreen agents are esters, most of the degradation products will be formed by ester hydrolysis. Dinunzio et al.(12) used RPLC to separate a mixture of the potential degradation products and their parent products (Table 1). This method

Table 1. Relative Retention of Sunscreen Compounds and Degradation Products

Compound	Type of Compound	Relative Retention
Anthranilic acid	Degradation product	0.30
p-Methoxycinnamic acid	Degradation product	0.31
4-(Dimethylamino) benzoic acid	Degradation product	0.35
Salicylic acid	Degradation product	0.41
Benzophenone-3	Sunscreen	0.60
2-Chloroanthracene	Internal standard	1.00
Octyl dimethyl PABA	Sunscreen	1.32
Menthyl anthranilate	Sunscreen	1.57
Octyl methoxycinnamate	Sunscreen	1.74
Octyl salicylates	Sunscreen	2.11

Column, Waters μBondapak C18; eluent: THF-acetic acid-water (55:0.09:44.91 v/v). Reprinted with permission from reference 12.

was applicable in monitoring product stability since very little sample preparation was needed.

Biological Studies

Early sunscreens were easily removed from the skin by sweating or water activities. The sunscreens today are more lipid soluble; therefore, they attach to the skin better and are not as easily removed.(39)

The skin is made up of three layers as shown in Fig. 1, the epidermis (outer layer), the dermis (middle layer), and the subcutaneous layer. The outermost sublayer of the epidermis is called the stratum corneum and it is this layer the sunscreen would be applied to. The stratum corneum is permeable to lipid soluble molecules; the more lipid soluble a molecule, the greater it will be absorbed. Once chemicals pass the epidermis and the dermis they are absorbed into the circulatory system.(42) Since sunscreens are applied on wide areas of the skin, it is necessary for the effectiveness and toxicity of the sunscreen that the sunscreens penetrate very little.(39,42) The penetration of the products in the sunscreen agents is specially important with the knowledge that these compounds also contain photodecomposition compounds.

Jiang et al,(39) Lazar et al.,(42) and Potard et al.(43) all did in vitro studies using sunscreen agents and biological media. All three studies included the use of RPLC on C18 columns with UV detectors.

Jiang et al.(39) assessed Escalol 507, Parsol MCX, Parsol 1789, oxybenzone, and octyl salycilate in cosmetic products, bovine serum albumin (BSA),

and human plasma. In laboratories, the action of sunscreen on skin is often mimicked using a diffusion cell with sunscreen on one side of the membrane, which represented the skin in the middle and BSA on the other side as the receptor fluid. The sunscreen is absorbed through the membrane into the BSA, similar to the absorption of the UV filters through the skin into the blood. Analyzing sunscreens in BSA, as well as human plasma, shows that this method would be useful for both in vitro and in vivo studies (Fig. 17). BSA and plasma was spiked with sunscreens into the plasma and BSA and recovery studies were carried out

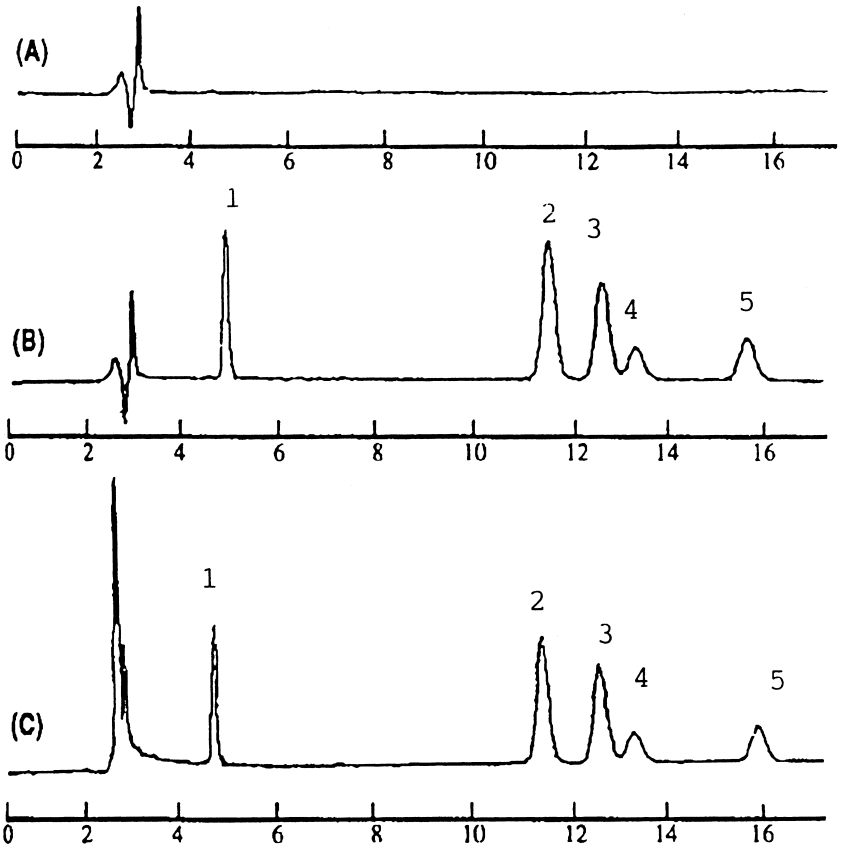


Figure 17. Chromatograms of blank (A) 2% BSA blank; (B) an extract from 2% BSA in Phosphate buffer; (c) plasma. Peaks: 1: Oxybenzone; 2: Escalol 507; 3: Parsol MCX; 4: Parsol 1789; 5: octyl salicylate. 4 μm C_{18} column; eluent: methanol-water (88:12); flow rate 1.0 mL/min; column temperature ambient (adapted with permission from Ref. 39).

with detection at 315 nm. This method was shown to be useful for sunscreen analysis as well as absorption studies.

Lazar et al.(42) used several types of emulsions containing octyl methoxycinnamate and butyl methoxy dibenzoylmethane for their in vitro percutaneous absorption study. They created a diffusion cell with the stratum corneum in contact with the sunscreen solution and the dermis in contact with the receptor fluid (BSA, NaCl and an ether). The receptor fluids were analyzed using detection at 325 nm and they were able to evaluate the amount of UV filters present in the various samples. The degree that the chemical filters penetrate into the skin is dependent on the type of emulsion and the vehicle properties. In fact, Lazar et al.(42) found oily external phases are the best sunscreen preparations, since they have low penetration rates for the UV filters and stay on the stratum corneum rather than diffusing through the epidermis.

Potard et al.(43) studied in vitro percutaneous penetration of octyl methoxycinnamate, benzophenone-3, benzophenone-4, octyl triazone, and octocrylene. Their goal was to establish standard operating procedures for analyses in various skin samples. Human skin was used to make a diffusion cell with a receptor fluid containing BSA and three salts. After the sunscreens were given time to absorb, the layers of the skin were analyzed using UV detection at the maximum absorbance of each product. The amounts of the UV-filters in the different layers of the skin were quantified. Potard et al.(43) also mentioned that the skin of different people reacts differently and, therefore, penetration varies among individuals as well.

Experimental

Active and inactive ingredients in formulations vary so much that finding a universal analytical method for these compounds is difficult.(10). Since sunscreen formulations are complex, UV spectroscopic methods do not allow identification or quantitation.(15). The chemical filters must first be separated by extraction and/or chromatography. Extraction removes the sunscreens from their base, while chromatography separates the mixtures into their individual components.(10) Stokes et al.(24) who did photostability analyses found transmission spectroscopy and separation techniques should be used together. The transmission techniques are affected by optical factors, such as the UV absorbing properties of the products formed. Meanwhile, with the separation techniques, changes in the UV absorbing properties after irradiation cannot be quantified.

Thin layer Chromatography (TLC) was at one time a popular method for the analysis of sunscreens. It is simple and inexpensive; therefore, it is readily available for most labs. As technology progressed and the labor-intensive method

of TLC became less desirable, gas chromatography (GC) and high performance liquid chromatography (HPLC) became popular for separating components in sunscreen formulations. GC tends to be widely used because it has good separation power; however, inactive ingredients in cosmetics sometimes give peaks that overlap with the sunscreen agents when using this method. HPLC has some advantages in that it is applicable to all sunscreens, even salts, and there is no need to derivatize the compounds.(10,35)

In biological studies with sunscreens, Lazar et al.,(42) who used UV spectroscopy, gas chromatography with a flame ionization detector, and HPLC-UV, found the HPLC method to be best. UV spectroscopy alone was not sufficiently specific. The GC required long sample treatment that lowered the yield. In addition, the temperature was too low for good chromatographic results; therefore, the detection limits were not adequate. The HPLC was found to be quicker and easier, with better detection limits than the GC.

A major problem with HPLC is that the compounds do not always separate completely in one run. It is often necessary to have more than one HPLC chromatographic run or the use of another separation method such as GC to complete the separation, and important isomers are at times neglected.(15) A simple HPLC run does not give enough information, since the filters must still be identified and quantified. In GC and HPLC, the retention times can be compared with a standard to obtain a tentative identification.(25)

Treatment/Extraction

Methanol (or acidified methanol), or methanol and chloroform have been used in a number of studies to dissolve or extract the sunscreen agents from samples.(10,11,28,29,30,32,35,37,39,43) De Orsi et al.(40) used methanol-aqueous sodium hydroxide for their extraction. Methanol is widely used because it does not remove lipids or carbohydrates during the extraction of sunscreens. Tan et al.(37) found that using large amounts of methanol to dilute samples of lotion and lipstick that they analyzed, caused most of the inactive ingredients to precipitate, resulting in better chromatograms and preserving the life of the column. Chloroform is good as a general-purpose solvent. It has a high density, which makes it easy to remove in liquid-liquid separations and does not react readily with the chemicals to be analyzed.(25)

Other alternatives chosen for solvent extraction were tetrahydrofuran (or acidified THF) with acetonitrile or with methanol and TFA.(12,15,32) Ethanol (1,35,42) and isopropanol(36,43) were used more infrequently for extraction.

Most of the sample treatments involved solvent extraction with sonication and centrifugation. These treatment processes are laborious, so Scalia(34) used a

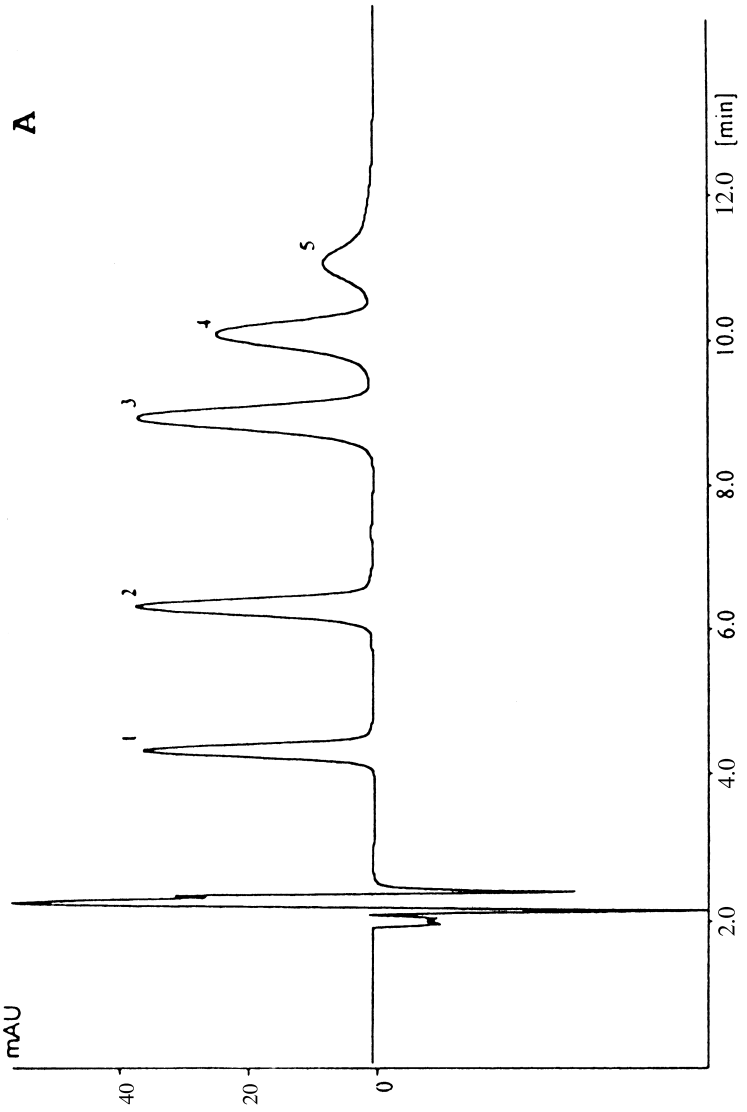
supercritical fluid chromatography technique (SFC) and obtained good separation (Fig. 18). This technique works well when extracting organic compounds from solid and semi-solid bases. Extraction with carbon dioxide is cost effective and safe, as well as easy to use, since the solvent properties can be modified by temperature or pressure. Very little sample manipulation and much less solvent is required. In another method of extraction, a micellar solution of sodium dodecyl sulfate (SDS) and triethyl amine at pH 3 was used to dissolve the components of the cosmetic formulations. Tomasella et al.(44) found that by using this method they were able to perform direct HPLC analyses of the UV filters. Shih and Cheng(45) used microwave-assisted extraction (MAE) to remove the sunscreen agents from the cosmetic products, since this technique provides high recoveries in short times. MAE is often used for biological and environmental samples. It is faster than liquid-liquid extraction and uses less solvent. The normal phase procedure out lined by Shaath et al.(10) used methylene chloride, a good general-purpose solvent for extraction. It is easily evaporated and quite non-reactive(25) (Table 2).

Separation

Almost all of the papers reviewed use RPLC, which is ideal for the analysis of the sunscreen formulations since they usually have a water-resistant base. Shaath et al.(10) outlined one of the only normal phase procedures. While normal phase separations on silica gel are good for some sunscreen separations, there are problems with retention time reproducibility. The separation is adsorption based and, therefore, the silica gel is affected by the previous sample(25). The column of choice in most cases is a C18 type. Tomasella et al.(44) used a C8 column as did Meijer and Loden(1) and Vanquerp et al.(38) who did stability tests. With C-18 stationary phases there are no column memory effects and, therefore, retention times are more consistent.(25)

Both isocratic and gradient elution have been used. Solvent gradients allow for a wide range of polarities of the ingredients,(25) but Wang et al.(35) did not recommend solvent gradients for routine analysis because of the expense and the length of time required for analysis.

The mobile phases used for the reversed phase analysis were mostly methanol and/or acetonitrile with or without water. Since sunscreens usually have a UV maximum in the 270-360 nm region, methanol and acetonitrile are excellent for use as mobile phases, since they have very high transmittance in those areas and mix well with water when degassed.(25) In certain cases, THF was used along with the methanol and/or acetonitrile.(12,18,31,34) Dinunzio et al.,(12) used acidified THF after selectivity tests. These selectivity tests with



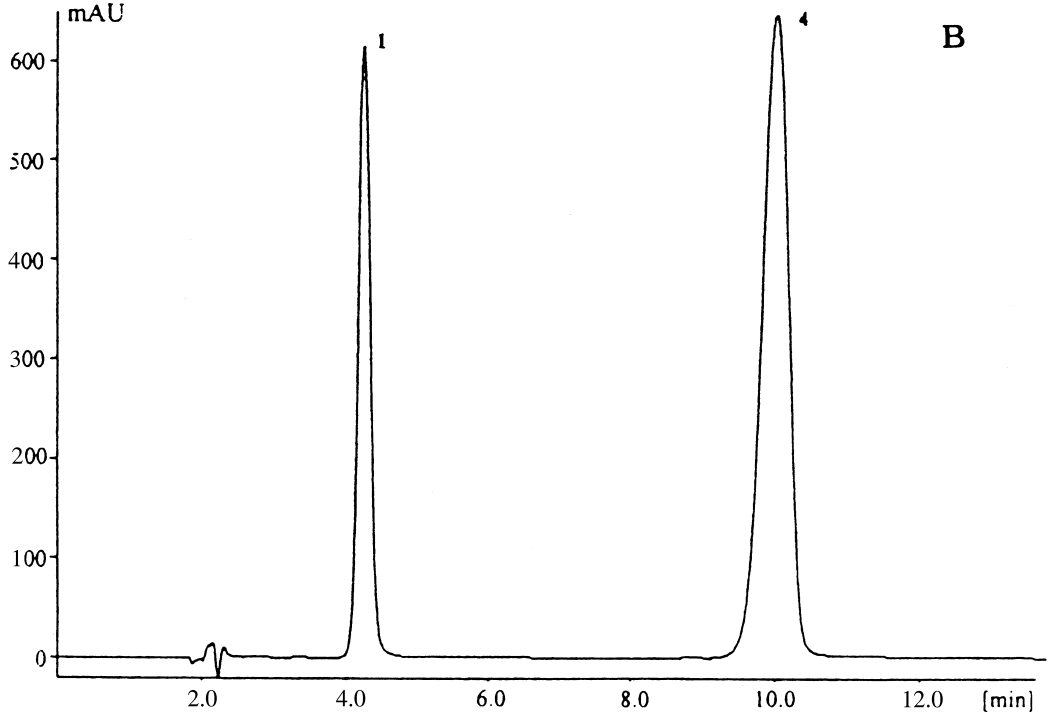


Figure 18. HPLC separation of a standard mixture of sunscreen agents. 1: 2-hydroxy-4-methoxybenzophenone; 2: 2ethylhexyl p-dimethylaminobenzoate. 3: 4-methylbenzylidene camphor; 4: 2-ethylhexyl p-methoxycinnamte. 5: 4-tert-butyl-4'-methoxydibenzoylmethane. HPLC diagram of a lipstick purified by SFE peak identification as in (A). Both conditions: 5 μ Hypersil BDS phenyl column; Eluent methanol-acetonnitrile-THF-water (45:10:10:35 v/v) containing 0.5%(v/v) acetic acid; Column temperature 40°C (adapted from Ref. 34).

Table 2. Summary of Conditions and Methods for Analysis of Sunscreens

Samples	Sample Preparation	Separation Type	Column	Mobile Phase	Buffer/Acid	Detection	Ref.
Sunscreen Emulsions	Ethanol extraction	RP Gradient	5μ C8	Methanol-water		UV 325nm	1
Standard Method	Methanolic extraction	RP Isocratic	10μm C18	Acetonitrile-water		UV 313nm	10
Standard Method	Water-methylene chloride & isopropanol-methylene chloride extractions	NP Isocratic	5μ silica	Hexane-methylene chloride	Acetic acid		10
Sunscreen	Acidic methanol extraction	RP Isocratic	5μ C18	Acetonitrile-water		UV 254nm	11
Sunscreens	THF, acetonitrile extraction	RP Isocratic	10μm C18	THF	Acetic	UV 311nm	12
Sunscreen	THF, TFA, methanol extraction	RP Gradient	5μ C18	Methanol-acetonitrile-water		PDA 325 & 360nm	15
Cosmetics & Suntan	THF extraction	RP Isocratic	5μm C18	Methanol-THF-water		UV 325nm	18
Suncreams	Acidic methanolic extraction	RP Gradient	10μm C18	Acetonitrile-water	Perchlorate & Tetra methyl ammonium chloride	UV 274nm 295nm	28

Creams	Methanol-chloroform organic extract	RP Isocratic & gradient	10μm C18	Phase A Acetonitrile-water Phase B Methanol-water	Phase A Perchlorate & Tetra methyl ammonium chloride	UV 280nm 313nm	29
Suntan Preparations	Sulphuric acid, methanol extraction	RP Gradient	10μm C18	Acetonitrile-water	Perchloric/ perchlorate	PDA 311nm	30
Lotions, Creams	Acidic methanol dissolution	RP Gradient	5μ C18	Buffer-acetonitrile-THF	Tetra butyl ammonium hydroxide-ammonia Citric acid	PDA 240–400nm	31
Cosmetic	Acidic THF extraction	RP Gradient	5μm C18	Methanol-Acetonitrile-water	Acetic	PDA 292nm	32
Lipstick, Cream	SFE	RP Isocratic	5μ C18	Methanol-acetonitrile-THF-water	Acetic	UV 320nm	34
Suncream, Lotions, Lipstick	Methanol dissolution Lipstick-chloroform-methanol organic extraction	RP Gradient & isocratic	5μ C18	Organic phase Acetonitrile-water & Aq. phase	Acetic	UV 286nm	35
Sunscreens	Isopropanol dissolution	RP Isocratic	5μ C18	Methanol-water	Acetic	UV 308nm	36

(continued)

Table 2. Continued

Samples	Sample preparation	Separation type	Column	Mobile phase	Buffer/Acid	Detection	Ref.
Sunscreens & Lipstick	Methanolic extraction	RP Isocratic	10μm C18	Methanol-acetonitrile		UV 254nm	37
Filters in Solvent	Water or ethanol dissolution	RP Gradient	5μ C8	Methanol-water	Acetic	UV 300nm	38
Sunscreens & Biological Samples	Methanol extraction	RP Isocratic	4μ C18	Methanol-water		UV 315nm	39
Oil in Water Cosmetic Formulations	Methanol-aq Sodium Hydroxide extraction	RP Isocratic	5μ C18	Acetonitrile-water	Perchlorate	PDA 263nm	40
Emulsions & Biological Samples	Ethanol extraction	RP Isocratic	5μ C18	TFA/acetonitrile/ methanol		UV 325nm	42
Skin Layers	Isopropanol & methanol: water extractions	RP Isocratic	4μ C18	Methanol-water	Acetic acid or ortho-phosphoric	UV λ_{max} each product	43
OTC Sunscreen Preparations	Isopropanol, mobile phase extraction	RP Isocratic	10μ C18	SDS isopropanol		UV 254,300nm	44
Creams	MAE	RP Isocratic	5μ C18	Acetonitrile-water		UV 300nm	45

THF, acetonitrile, and methanol showed that THF allowed the complete separation of octylsalicylate and octyl methoxycinnamate, while the acetonitrile and methanol did not. Acids and acid buffers are known to reduce peak tailing, so Dinunzio et al. used acetic acid and found a significant reduction in peak tailing (Table 3). Other mobile phases were acidified with acetic acid(12,32,35,36) while some were acid buffered. The buffers were used because most of the reverse phase columns have silica backbones that are only stable at or below pH7 and the ionization equilibria must be suppressed.(25)

Tomasella et al.(44) used SDS-isopropanol for their mobile phase, with SDS as the micellar reagent. With the normal phase HPLC, Shaath used acidified hexane and methylenechloride.

Detection

The detectors used were either UV detectors(1,10,11,12,18,28,29,34,35, 36,37,38,40,42,43,44,45) or diode array detectors(15,30,31, 32,39). The wavelengths chosen varied between 200-420 nm, depending on the maximum absorbance of the compound being analyzed. Some mixtures of sunscreens were analyzed with a wavelength in the region of 320 nm-325 nm. Several absorbers had significant absorbance in this region and, therefore, a single wavelength can be used to analyze all the filters in the mixture.(1,15,18,34)

Data Analysis Using Multi-Component Analysis (MCA)

Analysis of sunscreens involves work with complicated matrices that often give overlapping peaks in the chromatograms.(46) In certain instances, it may not possible to achieve baseline resolution or the amount of time needed to

Table 3. Effect of Acid on Peak Symmetry

Acetic Acid (%, v/v)	Tailing Factor
0.00	5.34
0.03	1.64
0.12	1.08
0.25	1.00
0.37	1.00
0.49	1.00
0.61	1.00

Column, μBondapak C18; eluent:, 65%(v/v) THF-acetic acid-water. Reprinted with permission from reference 12.

achieve it is not practical. MCA can be used to achieve quantitative results without baseline resolution, after analysis by photodiode array detection, and to analyze the purity of the components. MCA uses reference spectra and applies mathematical manipulations based on the hypothesis that any unknown spectrum is a combination of the reference spectra. Excoffier et al.(46) used a mixture of sunscreen agents; menthyl-p-aminobenzoate. 2-ethylhexyl p-methoxycinnamate, 2-ethylhexylsalicylate, and oxybenzone (2-hydroxy-4-methoxybenzophenone) and analyzed them using HPLC-UV-DAD. The sunscreen agents were completely separated using a C18 column and gradient elution with an acidified methanol:water mobile phase (75:25) to (95:5) in 8 minutes. They also achieved complete separation on the same column using isocratic elution with methanol:water (85:15). The detection was performed using a diode array detector over 190-369 nm. Using the same conditions, they obtained reference spectra of the individual compounds. To achieve partial resolution they performed the separation isocratically using 100% methanol, and also obtained reference spectra under these new conditions. For zero resolution, the column was replaced by 50ft of coiled stainless-steel tubing with 100% methanol mobile phase and the mixture, as well as the individual components, were injected. MCA was applied to the data obtained. The unresolved chromatographic data were quantified, identities of peaks were confirmed, and the purity of the components was evaluated.

CONCLUSIONS

Armed with the knowledge that UV radiation can do serious damage to the skin and health, the public is dependent, more than ever, on sunscreens to protect them. With the widespread use of sunscreens and the wide variety of formulations that now exist, analysis and quality control of sunscreens has become even more important.

Several successful HPLC methods have been developed for the quality control aspects of the sunscreen industry; ensuring that maximum concentrations are not exceeded and that forbidden contaminants are not included.

Studies of the photostability of sunscreens are also a necessity, since knowledge of shelf life, photoproducts, and breakdown products is important to keep the sunscreen formulations safe for use. As a result, several successful methods have been developed using HPLC to determine and quantify these photoproducts in cosmetics. Several compounds are known to degrade after irradiation with UV light. Investigation into the conditions that retard the degradation process would useful in choosing optimal ingredients in the formulation; it may also lead to the development of photostabilizers for sunscreens.

In addition, in vitro percutaneous studies are of importance to estimate the penetration of the sunscreen filters into the body. Although, penetration depends

on formulation and skin type, it is important to have knowledge of the penetration power of breakdown products of the formulations that are frequently applied to skin. More work needs to be done on the nature of the UV degradation products, the conditions under which they absorb into the skin, and the effects they have on biological systems.

The cosmetic industry must do further work in the area of photostability and percutaneous absorption, in order to develop sunscreens that are safe for the body, as well as, protect the skin.

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