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Determination of *p*-Amino Benzoic Acid and *p*-Hydroxy Methyl Benzoate in Sunscreens by HPLC

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A selective, sensitive method designed for the determination and isolation of *para*-amino benzoic acid (PABA) and its derivatives in sunscreen by HPLC. The researches confirm that sunscreen must be free from *para*-amino benzoic acid (PABA) and *p*-hydroxy methyl benzoate because these compounds cause allergy and phototoxic, photoallergic, damage the DNA and cause cancer. We have studied of 54 sunscreens by HPLC, imported and local, and found the average value of PABA for both local or imported is null for 12 imported products and 20 local. While the average value in range [0, 0.1] to 3 imported products and 4 local and the average value of *p*-hydroxy methyl benzoate is null also for 2 imported products and one for local products. Finally we notice the sum of averaged value of *para*-amino benzoic acid (PABA) and *p*-hydroxy methyl benzoate (PHMB) is far less for some imported products which we already analyzed.

Key Words: Sunscreen, High performance liquid chromatography, *p*-Amino benzoic acid, *p*-Hydroxy methyl benzoate.

INTRODUCTION

The allure of a golden tan has paled somewhat in recent years. The growing awareness of the role of UV light in skin cancer and premature wrinkling has led hosts of modern day sun-worshipers to join the burn-and-peel set in donning copious amounts of sunscreen before venturing outdoors. To some, these miraculous lotions represent freedom from blistering skin and sweltering clothes, whereas to others, they hold the promise of youthful skin well into the future. The proliferation of sunscreen is trully a monument to the use of chemistry to improve the lives and recreation of millions[1]. So the first UV absorber to be widely used was *para*-amino benzoic acid (PABA) which was popular in sunscreen as early as the 1920s[2]. PABA is good absorber of UVI_3 and is relatively cheap which has significant solubility in water. In the mid-1970 with the development of PABA esters, in which the carboxylic acid group of molecule has been functionalized with long chain aliphatic alcohol, and PABA esters were also much less irritating to the skin[3]. Dobak *et al.*[4] found that the contact dermatitis

and photoallergic contact dermatitis with different sunscreens can occur at the same time and can be explained by a combination of cross-reaction and coupling allergy. But patients who are sensitized to some compounds like aniline, procaine, sulfonamides may develop cross-sensitization to a PABA sunscreen, and patients sensitive to PABA derivatives can also be sensitive to PABA. A report concluded that 14 out of 17 sunscreens containing PABA may actually cause skin cancer. This furthermore indicates that PABA causes genetic damage to the DNA in the skin[5] and that most high factor sunscreens can stimulate the formation of cancer cells[6] The work has shown that some of the agents that cause phototoxicity can also cause photoallergy, including one of the most common sunscreens PABA[7], however in recent years a report in the news media stating that PABA, padimate-O could decompose to give an N-nitrosamine degradation product, because some N-nitrosamines were known to be potent carcinogens; this report caused a flurry of alarm among sunscreen users. Nevertheless, this issue hurts the reputation of PABA-based sunscreen and their departure from the field[8]. Now-a-days it is difficult to find a sunscreen that contains *para*-amino benzoic acid or *para*-amino benzoic esters[9].

EXPERIMENTAL

A Shimadzu LC-10A series HPLC was used. All analyses were performed on a column shim pack CLG-ODS, Erbasil (15 cm × 4.6 mm). Detection was performed by means of SPD 10A/10AV UV-visible spectrophotometer detector. Injections were made by SIL-10A AUT injector. Peak heights were recorded with a strip chart electronically integrated with a Hewlett reporting integrator.

Acetonitrile (ACN) was used as obtained from Scharlau Chemie; perchloric acid, tetramethyl ammonium chloride and methanol, and were all used as obtained by the Sigma Co., St. Louis, Mo.

The mobile phase was 10 mM perchloric acid and 5 mM tetramethyl ammonium chloride $(CH_3)_4NCl$ at pH = 3. A flow rate of 1.2 mL/min was used. The injection volume of each sample was 10 μL. The temperature was 50°C.

Sample preparation: Take 1 g of sunscreen cream sample in a beaker, add equivalent amount of sodium chloride, 1 mL of H_2SO_4 (1 M) and nearly 15 mL of methanol; then transfer the contents of beaker to ultrasonic apparatus for 15 min at 50°C. Now transfer the part which dissolved (filtrate) to measuring flask 100 mL and the other part which was not dissolved from sample treated with the amount of methanol several times to arrive to complete extraction. Then filtrate with special filter for HPLC and keep in test tube to use.

Preparation of PABA: Standard *para* amino benzoic acid was prepared 0.2 g per 100 mL as stock solution and serial dilutions 20, 2.0, 0.20, and 0.04 microgram per mL.

Chromatographic procedure

The intral isocratic conditions was modified[10]. By increasing the flow rate beginning at 0.2 mL/min, 0.4 mL/min and until 1.2 mL/min. At this flow rate the time of injection equals zero and the concentration of acetonitrile equals 18% and after 10 min the concentration of acetonitrile rose to reach 50% and then the buffer solution of mobile phase is also 50%. Under these conditions *p*-amino benzoic acid eluted in 2.77 min and *p*-hydroxy methyl benzoate in 8.28 min. Detection was at 274 nm throughout and 10 µL of sample was injected for all samples.

Flowrate program

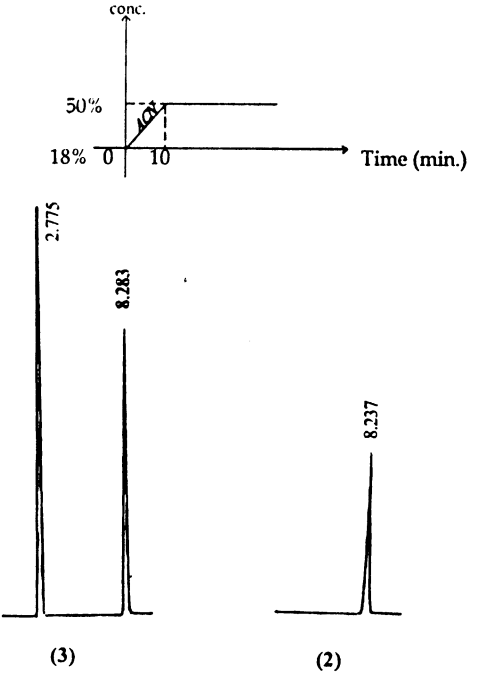
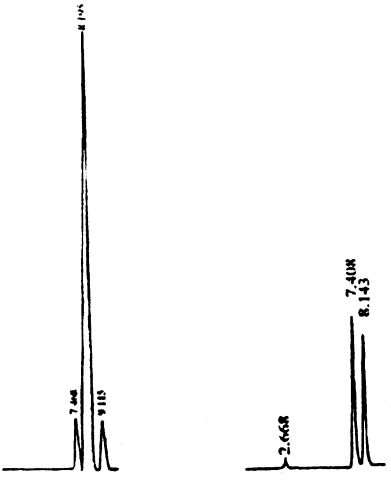


Fig. 1. Chromatographic separation on CLG ODS Erbasil (15 cm × 4.6 mm), mobile phase acetonitrile-buffer solution $[(CH_3)_4NCl + HClO_4]$ at pH = 3, flow rate 1.2 mL/min, detection UV at 274 nm, peak identification: (1) PABA, (2) PHMB, (3) mix. [PABA + PHMB].

Number	Time	Area	Conc. (µg/mL)	Name
1.	2.772	437500	20.0000	PABA
2.	8.237	243261	20.0000	PHMB
3.	2.775	425268	19.4154	PABA
	8.283	241542	20.5643	PHMB
	Total	666810	39.9697	

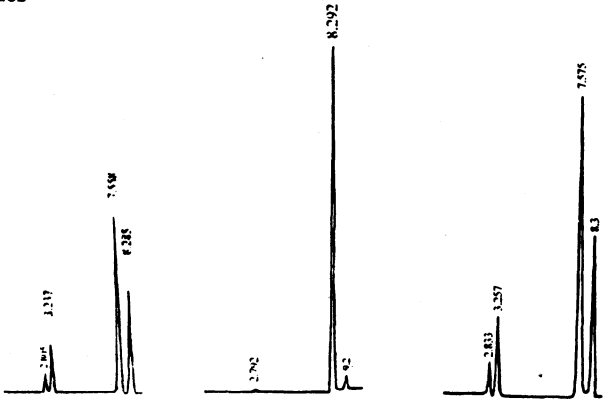
The imported samples



(4) (5)

No.	Time	Area	Conc. (μg/mL)	Name
4.	8.195	641842	53.4868	PHMB
5.	2.668	10980	0.4695	PABA
	8.143	145376	12.1372	PHMB
	Total	156356	12.6067	

The local samples



(6) (7) (8)

Number	Time	Area	Conc. (μg/mL)	Name
	2.805	15581	0.7187	PABA
6.	8.285	95113	7.7771	PHMB
	Total	110694	8.4958	
	2.792	1119	0.0526	PABA
7.	8.292	331706	27.4658	PHMB
	Total	332825	27.5184	
	2.833	17731	0.8339	PABA
8.	8.300	99326	8.2243	PHMB
	Total	117057	9.0582	

TABLE-1
THE VARIOUS IMPORTED SUNSCREENS FREE OF (PABA) AND CONTAINING VARIOUS CONCENTRATIONS OF *p*-HYDROXY METHYL BENZOATE (PHMB)

Prod.	Sun protection factor (SPF)	Conc. (PABA) (μg/mL)	Conc. (PHMB) (μg/mL)
Ro.	25	0.00	3.34
Ro.	40	0.00	19.41
Qta.	—	0.00	0.00
N.V.	—	0.00	55.11
Clar.	35	0.00	24.24
Natu.	90	0.00	23.89
Gohn.	—	0.00	18.35
Atoe.	4	0.00	18.81
Spec.	30	0.00	12.26
Yard.	30	0.00	0.55
Colla.	—	0.00	114.05
Photo.	100	0.00	16.80

Prod. = Name of the manufacturing Co.

TABLE-2
THE VARIOUS CONCENTRATIONS FOR (PABA) AND (PHMB) COMPOUNDS FOR THE SEPARATED SAMPLES EVALUATED BY (HPLC) FOR THE IMPORTED SUNSCREENS FROM VARIOUS COMPANIES

Prod.	Sun protection factor (SPF)	Conc. (PABA) (μg/mL)	Conc. (PHMB) (μg/mL)
F.A.L.	—	0.09	18.26
F.A.L.	—	0.33	3.85
Ola.	—	0.05	0.00
Ant. Xl.	60	0.23	16.02
Ave.	60	0.47	12.14
Hyd.Leg.	—	0.09	10.94
Gale.	40	0.19	12.73
Pon.	—	0.22	16.77
Luts.	30	0.18	24.72
Acen.	4	0.23	17.26

Prod. = Name of the manufacturing Co.

TABLE-3
THE VARIOUS LOCAL SUNSCREENS COMPLETELY FREE OF THE (PABA), WHILE CONTAINING VARIOUS CONCENTRATIONS OF THE (PHMB) COMPOUND

Prod.	SPF	Conc. (PABA) (μg/mL)	Conc. (PHMB) (μg/mL)
Most.	15	0.00	20.77
Pref.	8	0.00	16.84
Pref.	15	0.00	18.97
Pref.	35	0.00	19.04
Alle.	8	0.00	12.19
Alle.	19	0.00	10.18
Alle.	30	0.00	21.44
Alle.	30	0.00	12.11
Cood.	25	0.00	13.64
Izi.	100	0.00	11.52
N.F.	—	0.00	8.00
Hell.	40	0.00	9.46
Gell.	8	0.00	21.90
Gell.	15	0.00	23.15
Gell.	20	0.00	22.20
Gell.	30	0.00	18.87
Gell.	45	0.00	19.07
Gell.	60	0.00	16.95
Gaya.	40	0.00	15.42
F.A.L.	—	0.00	0.00

TABLE-4
THE VARIOUS CONCENTRATIONS OF (PABA) AND (PHMB) COMPOUNDS FOR ISOLATED AND EVALUATED SAMPLES BY (HPLC) METHOD FOR THE LOCAL SUN-SCREENS OF VARIOUS COMPANIES

Prod.	SPF	Conc. (PABA) (μg/mL)	Conc. (PHMB) (μg/mL)
Most.	8	0.05	27.47
Most.	25	0.83	8.22
Most.	30	0.72	7.78
Mess.L.	8	1.45	6.28
Mil.	8	0.25	24.16
Alle.	30	0.06	11.77
Alle.	72	0.06	11.15
Izi.	20	0.35	7.44
Izi.	45	0.40	9.30
Unit.	45	0.32	9.21
Jor.	—	0.03	19.14
Ziro.	30	0.24	16.97

SPF = SUN protection factor.

Prod. = Name of the manufacturing company.

RESULTS AND DISCUSSION

We studied 54 samples of imported and local sunscreen products to separate and determine the (PABA) and (PHMB); we found that 19 samples from 32 local products were free exactly from PABA, but contain the derivatives of PABA which are called *p*-hydroxy methyl benzoate (PHMB) to concentration range (8.00 μg/mL, 12.19 μg/mL, 18.97 μg/mL and 23.15 μg/mL) Raeng (8.00–23.15) μg/mL. While 13 local samples contain PABA with different concentrations for **Jor.** Sample was 0.030 μg/mL while in **Alle.** Sample was 0.060 μg/mL followed by **Ziro.** Sample with concentration of 0.2405 μg/mL, an **Izi.** Sample with 0.4047 μg/mL, and **Most.** Samples are 0.8326 μg/mL while the methyl para hydroxy benzoate was found to contain less concentration in **Most.** Samples of 7.7811 μg/mL but the remaining samples contain range of (8.2217–274680) μg/mL. At last we, found only one sample from these local samples, free completely from both PABA and methyl para hydroxy benzoate. Consequently, of the 22 products samples imported we found 50% were free from PABA while **Ola.** Sample contained less concentration value of 0.0509 μg/mL followed by **Hyd.** Sample with 0.0894 μg/mL and 32% of these samples were concentration between range of PABA from (0.1815–0.3303) μg/mL and these concentrations were very low. But we found only two samples which wre **Colla.** and **N.V.** were free from PABA and contain high concentrations of PHMB with 114.0052 μg/mL and 55.3639 μg/mL while we found 31% from imported samples have concentration range between 16.8000–23.8900 μg/mL. Finally, we realized that 39% from imported samples have PABA in range of 0.4700 μg/mL while that of PHMB was 12.1400 μg/mL. Consequently, it appears that little products sunscreen haven't PABA can't cause any irritation to the skin and are of high quality, but many of these products of native or imported sunscreens contain PABA and its derivatives so can both be irritant allergic, phototoxic, and photoallergic reactions have been reported following the use of chemical sunscreens[3] chemical sunscreens are wavelength-selective with specific absorption spectrum. The UVA sunscreens absorb within a limited spectrum, usually at shorter wavelength of UVA, on the other hand, are affective in absorbing the entire UVB spectrum. We conclude that those studied local or imported samples of sunscreens characterize different sun protection factors (SPF) 8, 15, 20, 25, 30, 35, 45, 72 and 100 we found no relation between the concentration of PABA and its derivatives PHMB. No matter which sunscreen is used, the degee of protection is only partial[3].

Conclusions

The skin is a carefully designed interface between our bodies and the outside world. Sunlight threatens the health of the skin. Normal skin is highly variable in its ability to resist sun damage. Natural skin pigmentation is its main protection. The term photosensitivity refers to any increase beyond what is considered normal variation[11]. The above results and the research confirm that PABA and its derivatives can cause photoallergy, phototoxicity, and these products local and imported must be free from PABA and its derivatives.

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