Journal of Food and Drug Analysis

Volume 7 | Issue 1 Article 7

Studies on analytical method for determination of fluorescent whitening agents in paper containers for food

Journal of Food and Drug Analysis
1999. 7(1) : 53-63

藥物食品分析
第七卷 第一期

Studies on Analytical Method for Determination of Fluorescent Whitening Agents in Paper Containers for Food

MAI-LING JAO*, MEI-ING LIAO, CHUN-TZU HUANG,
CHIEU-CHEN CHENG AND SHIN-SHU CHOU

Division of Food Chemistry, National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, 161-2, Kuen Yang Street, Nankang 115, Taipei, Taiwan, R.O.C.

ABSTRACT

The method of determination of fluorescent whitening agents (FWA) in paper containers for food was studied. Samples were assessed in accordance with official procedures. The treated gauze was extracted by dimethylformamide, evaporated to dryness, and then analyzed by HPLC. The extracted FWAs were separated with Prodigy 5 μm ODS (2) column using acetonitrile: 0.01 M sodium dihydrogen phosphate (35:65) as solvent. For spectrofluorimetric detection, an excitation wavelength of 340 nm and emission wavelength of 430 nm were used. Applying the above HPLC method, fifty samples of paper containers for food were analyzed. No residue of FWA was detected in any sample .

Key words: **high performance liquid chromatography (HPLC), fluorescent whitening agents, paper containers for food.**

INTRODUCTION

Fluorescent whitening agents (FWAs), which are a class of synthetic dyes, are capable of absorbing UV light at the range of 350~360 nm and emitting visible blue fluorescence at 420~450 nm. Using these dyes, not only the bluish hues in white color are compensated but also the intensities of visual reflection are enhanced. In terms of whitening mechanism, FWAs optically increase the feeling of whiteness but do not show any bleaching effect, a chemical destruction of pigment[1]. Because of these properties, FWAs have been widely used in textile, paper-making, soap, and detergent industries. FWAs are categorized into six groups by their basic chemical structures. They are (1) stilbene, (2) coumarin and quinolone, (3) pyrazoline,(4) naphthalamide, (5) benzoxazole and benzimidazole, and (6) distyrylbiphenyl. Many derivatives from above basic structures have been found and most of which are from stilbenes. FWAs are safe due to their low toxicity[2]. The acute toxicity study showed the LD_{50} of FWAs were higher than 7000 mg/kg. Subacute and chronic toxicity studies as well as other special toxicity tests indicated no tumor and intoxification symptoms occurred. Besides, no data showed the immigration effect on derma when human body exposed to the textiles treated with FWAs.

Correspondence to: Mai-Ling Jao

Accepted for Publication: Oct. 14, 1998

FWAs as food additives are prohibited although they are low in toxicity[3]. For chemicals serving as food additives, the safety and necessity issues need to be addressed. However, FWAs are mainly used as dyes rather than food additives. They are, therefore, restricted from being used in food. Furthermore, FWAs are not allowed to be used in food packages or containers for fear of contaminating food matrix. According to the regulations of food hygiene in Japan, FWAs are restricted as additives in any packaging materials that may come in contact with foods[4]. Therefore, in Japan FWAs are strictly prohibited from being used in paper containers such as paper plates, paper cups, and meal boxes.

Qualitative analysis of FWA was routinely performed using a spectrofluorometric method in accordance with Chinese National Standard[5] and a method announced by the Department of Health [6]. Test sample was soaked in ammonia water in which a gauze was placed to absorb the compounds of interest. The treated gauze was adjusted to pH 3~5 followed by a heating process prior to spectrofluorometric detection at UV 365 nm. This method, however, only gives a qualitative data. The identification of individual FWA need to be further carried out by either Thin Layer Chromatography (TLC)[7-10] or High Performance Liquid Chromatography (HPLC)[11-15]. The purpose of this study was to search for an ideal analytical method based on TLC or HPLC. The developed method was expected to be an official method for the identification of FWAs in food containers. The commercial paper containers including paper plates, paper cups, and meal boxes were also sampled and analyzed for the FWAs residues. Analytical results could be a reference for the consumers and related authorities.

MATERIALS AND METHODS

I. *Source of Test Samples*

Fifty test samples including 20 paper plates, 25 paper cups, and 5 meal boxes were purchased from supermarkets and retailers in Taipei during October, 1996 and May 1997.

II. *Source of FWA Standards*

FWA standards were obtained from various sources as listed in Table 1 and were numbered as FB24, FB28, FB71, FB85, FB90, FB119, FB121, FB257, FB263, FB266, and FB351 based on a color index.

III. *Analytical Methods*

(I) *Thin-Layer Chromatography (TLC)*

1. *Device*

UV lamp (Chromato-VUE, C-70G, UV Viewing System) was a product of UVP Co. (USA)

2. *Reagents*

The reagent grade Ammonia water, hydrogen chloride, dimethylformamide, acetone, benzene, diethanol amine, tetrabutylammonium iodide, acetylacetate, and 1,4-dioxane and LC grade acetonitrile and methanol were obtained from E. Merck Co. (Germany)

3. *Preparation of Sample Solutions*

A TLC method based on the Health Department Ordinance No. 762034 was adopted [6]. Test samples were soaked in ammonia water (pH 7.5~9) containing a FWA-free gauze (2 × 4 cm) at room temperature for 10 min. The ammonia water was then acidified to pH 3~5 with one drop of hydrogen chloride followed by a bath in water at elevated temperature for 30 min. The treated gauze was then washed with water and extracted with 50 ml of dimethylformamide for 5 min. Dimethylformamide phase was evaporated to dryness under vacuum. The extract was then reconstituted with 30% acetonitrile solution, which was then filtered through a 0.45 μm membrane. The test solution was thus prepared and ready for TLC analysis.

4. *Identification of FWA*

A silica gel plate (silica gel 60, Art.5748, E.

Merck, Germany) and six solvent developing systems were tested in this study according to the method of Ganz *et al*[8]. Developing solvent systems were as follows: (1) acetone: benzene: water: 25% ammonia water: tetrabutylammonium iodide (70/20/7/4/2, v/v/v/v/w); (2) acetone: water: 25% ammonia water: tetrabutylammonium iodide (90/4/10/2, v/v/v/w); (3) acetone: benzene: water: 25% ammonia water: tetrabutylammonium iodide (90/5/4/10/2, v/v/v/v/w) (4) 1,4-dioxane: benzene: methanol: 25% ammonia water (40/50/20/10, v/v/v/v); (5) acetone: benzene: 10% diethanol amine (90/25/10, v/v/v); (6) benzene: acetylacetate (95/5, v/v). After developing, the spots were located under UV light and those developed compounds were tentatively identified by comparing the relative mobility of samples with that of standards.

(II) *High Performance Liquid Chromatography (HPLC)*

1. *Device*

(1) Spectrophotometer: Spectronic 300 (Milton Roy Co., Oostende, Belgium)
(2) Fluorescence spectrophotometer: F-4500 (Hitachi Co., Tokyo, Japan)
(3) High performance liquid chromatography: Shimadzu LC-10AT solvent delivery system equipped with a Shimadzu SPD-10A UV-VIS detector and a RF-530 fluorescence detector (Shimadzu Co., Kyodo, Japan)
(4) Integrator: Shimadzu C-R4A (Shimadzu Co., Kyodo, Japan)
(5) HPLC column: Prodigy ODS-2, 5 μm 4.6 mm × 15 cm, Phenomenex Co. (CA, USA)

2. *Mobile Phase Preparation*

The mobile phase was prepared by mixing acetonitrile and 0.01 M sodium dihydrogen phosphate (35/65, v/v) followed by filtered through a 0.45 μm membrane prior to injection.

3. *Preparation of the Standard Stock Solution*

FB266 (100 mg) and other FWAs (10 mg of each) were individually weighed into a 100 ml of brown volumetric flask. A solution of acetonitrile: water (30/70, v/v) was then added to the volume. The standard stock solutions of FB266 with a concentration of 1000 μg/ml and other FWAs each with a concentration of 100 μg/ml were thus prepared.

4. *Preparation of Sample Solutions*

Sample preparations were carried out by TLC method as described above.

5. *Standard Curve Plotting*

The standard stock solutions of FB28, FB90, FB119, FB121, and FB257 were diluted to series concentrations of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 1 μg/ml; and those of FB24, FB85, and FB263 were diluted to the concentrations of 0.02, 0.05, 0.1, 0.2, 0.5, and 1 μg/ml. Stock solution of FB71 was diluted to the concentrations of 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, and 0.5 μg/ml; while stock solutions of FB251 and FB266 were diluted to a series concentrations of 0.001,0.002, 0.005, 0.01, 0.02, 0.05, and 0.1 μg/ml and 25, 50, 100, 200, 400, and 800 μg/ml, respectively. Ten μl of each diluted solution was injected to HPLC and each injection was carried out in triplicate. Eleven standard curves on the basis of peak areas vs. concentrations were thus plotted.

6. *Compounds Identification*

Ten μl of sample solution and standard solution was individually injected and compounds of interest were tentatively identified by comparing the retention times of unknown peaks to those of standard peaks. Analytical conditions for HPLC were as follows.

Column: Prodigy ODS-2, 5 μm 4.6 mm × 15 cm

Mobile phase: acetonitrile: 0.01 M sodium dihydrogen phosphate (35/65, v/v)

Detector: fluorescence detector with excitation wavelength at 340 nm and emission wavelength at 430 nm; and UV detector at 350 nm

Flow rate: 1.0 ml/min

RESULTS AND DISCUSSION

Table 1. Color index, chemical class, commercial name and source of fluorescent whitening agents

Color index	Chemical class	Commercial name	Source
FB 24[a]	Bistriazinylamino-stilbene	BB	Taiwan Sugar Industry Co., Ltd.
FB 28	Stilbene	–	Aldrich Chem. Co.
FB 71	Stilbene	DMS-X	Ciba-Geigh Co.
FB 85	Bistriazinylamino-stilbene	–	Formosa Chemicals & Fibre Corp.
FB 90	Bistriazinylamino-stilbene	BS	Kuang Chuan chemistry Co., Ltd.
FB 119	Stilbene	Blankophor REU	Bayer Taiwan Co., Ltd.
FB 121	Stilbene	Blankophor DCB Ultra Fine	Bayer Taiwan Co., Ltd.
FB 257	Coumarin	Blankophor ANR	Bayer Taiwan Co., Ltd.
FB 263	Triazinylamino-stilbene	Blankophor BRU Fluessing (liquid)	Bayer Taiwan Co., Ltd.
FB 266	Pyrazoline	Blankophor DCR (liquid)	Bayer Taiwan Co., Ltd.
FB 351	Distyrylbiphenyl	CBS-X	Ciba-Geigh Co.

[a]FB24: 4,4'-bis[(4-3-sulfoanilino-6-dihydroxyethylamino-1,3,5 triazin-2- yl)amino] stilbene-2,2'-disulfonic acid disodium salt.

FB 28: 4,4'-bis[(4-anilino-6-dihydroxyethylamino-1,3,5 triazin-2-yl)amino] stilbene-2,2'-disulfonic acid disodium salt.

FB 71: 4,4'-bis[(4-anilino-6-morpholino-1,3,5 triazin-2-yl)amino] stilbene-2,2'-disulfonic acid disodium salt.

FB 85: 4,4'-bis[(4-3-anilino-6-hydroxyethylamino-1,3,5 triazin-2-yl)amino] stilbene-2,2'-disulfonic acid disodium salt.

FB 90: 4,4'-bis[(4-anilino-6-methoxy-1,3,5 triazin-2-yl)amino] stilbene-2,2'-disulfonic acid disodium salt

FB121: 4-[3-(4-chloro-phenyl)-4,5-dihydro-pyrazo-l-yl]-benzene sulfoniamide.

FB351: 4,4'-bis(2-sulfostyryl)biphenyl disodium salt.

I. *Detection of FWAs in Paper Containers by TLC*

(I) *Selection of FWA Standards*

Of various FWA products, only one reagent grade FWA made by Aldrich Chem. Co (USA) was found. Seven imported FWAs were obtained from Ciba-Geigy and Taiwan Byer Co. Five FWAs were obtained from two chemical plants in Taiwan and four FWAs were commercially available. Eleven out of 17 FWAs were determined to be standards in this study after HPLC analysis of those 17 collected FWAs and an exploration of related references. According to the color index for synthetic dyes, these eleven FWAs were numbered as FB24 (Fluorescent Brightener 24), FB28, FB71, FB85, FB90, FB119, FB121, FB257, FB263, FB266, and FB351. Among 11 FWA standards, 7 of which were well-defined with complete chemical nomenclature, while the other 4 were in patent-pending and their chemical names and structures are not yet disclosed. Based on the color index, however, the structures of these 11 FWA standards were capable of being classified into 8 stilbenes, 1 coumarin, 1 pyrazoline, and 1 distyrylbipheynl as listed as Table 1.

(II) *Extraction of FWAs from Samples*

Health Department Ordinance No. 76203 [6] was used as the method for test of FWAs in 11 standards. Four solvent systems including (1) acetone, (2) methanol, (3) acetone: water: 25%

Table 2. Rf values of fluorescent whitening agents in TLC with six kinds of developing systems[a]

FWA	Rf Value									
	1		2		3		4		5	6
FB24	0.04		0.24		0.03		0.19		0	0
FB28	0.22	0.29	0.61	0.85	0.29	0.40	0.57	0.82	0	0
FB71	0.28	0.48	0.72	0.91	0.39	0.56	0.66	0.90	0	0
FB85	0.21	0.32	0.57	0.83	0.29	0.40	0.51	0.81	0	0
FB90	0.27	0.43	0.74	0.91	0.38	0.53	0.70	0.89	0	0
FB119	0.47		0.45	0.50	0.22	0.31	0.09	0.22	0	0
FB121	0.88		0.88		0.85		0.87		0.79	0.07
FB257	0.35	0.44	0.13		0.08		0.15		0	0.12
FB263	0.16	0.19	0.48	0.55	0.12	0.18	0.03	0.05	0	0
FB266	0.88		0.85		0.85		0.87		0	0.21
FB351	0.35		0.84		0.37		0.83		0	0

[a]1: Acetone:benzene:H_2O:NH_4OH:tetrabutylammonium iodide (TBAI)= 70 ml:20 ml:7 ml:4 ml:2 g.
2: Acetone:H_2O:NH_4OH:TBAI=90 ml:4 ml:10 ml:2 g.
3: Acetone:benzene:H_2O:NH_4OH:TBAI=90 ml:5 ml:4 ml:10 ml:2 g.
4: Acetone:benzene:10%diethanol amine=90 ml:25 ml:10 ml.
5: Dioxane:benzene:methanol:NH_4OH=40 ml:50 ml:20 ml:10 ml.
6: Benzene:ethylacetate=95 ml:5 ml.

Table 3. The excitation and emission of the highest fluorescent intensity of the fluorescent whitening agents in mobile phase

FWA	Excit. (nm)	Emiss. (nm)
FB24	336.4	430.0
FB28	335.4	431.6
FB71	336.4	431.0
FB85	332.0	431.2
FB90	334.8	429.8
FB119	333.6	428.2
FB121	362.6	452.6
FB257	358.6	445.0
FB263	335.0	429.0
FB266	363.8	441.8
FB351	345.0	426.0

ammonia water (90/10/5, v/v/v) and (4) dimethylformamide were used to extract solutions from the soaked gauze. Solvent selection was based on the method of Ganz *et al*[8], who used acetone and acetone: water: ammonia water mixture to extract FWA from mud and plant; the method of Micali *et al*[12], who used methanol for FWA extraction from detergents; and the method of Ito *et al*[7], who developed a FWA extraction procedure using dimethylformamide as extraction solvent. Results showed that FWA recovery was inconclusive when gauze was soaked in standard solution followed by solvent extraction with above four solvent systems. However, a solution with strong fluorescence was observed under UV 365 nm using acetone: water: ammonia water mixture as well as dimethylformamide as extraction solvents. Conversely, with the other two solvent systems, the solution with only slight fluorescence was detected. Acetone: water: ammonia water mixture resulted in foaming during vacuum rotary evaporation, therefore not suitable in this study. Dimethylformamide was the best choice because it could yield a satisfactory TLC as well as HPLC chromatogram, and it was used in this study.

(III) *Identification by TLC*

Table 2 presents the results of FWA analysis by TLC. Solvent systems 5 and 6 failed to develop 11 FWA standards, while the others were capable of developing those FWAs. The Rf values of

11 FWAs were close, implying that TLC method cannot be used for identification purposes but for determining if FWAs exist in test samples.

II. *Detection of FWAs in Paper Containers by HPLC*

(I) *Selection of Detection Wavelength*

Eleven FWA standards were dissolved in mobile phase and then scanned with fluorescemeter and spectrophotometer. The results are shown in Table 3 and Figure 1. In nature, FWAs exist in *trans* form. However, when exposed to sunlight, they tend to be tautermerized to *cis* form, which loses the fluorescence characteristic and can only be detected by UV detector[13, 15]. Eleven FWAs showed difference in maximum excitation and emission fluorescence wavelengths (Table 3). HPLC analysis achieved the best sensitivity with excitation wavelength at 340 nm and emission wavelength at 430 nm. Therefore those wavelength were used in this study (Figure 2). UV detection at 350 nm was also selected because FWAs gave the maximum absorbance at this wavelength as shown in Figure 1.

(II) *Optimization of the HPLC Conditions*

HPLC was performed based on the report of Micali *et al*[12], who demonstrated an ion par chromatographic method, and of Tsuji *et al*[13]. Reverse phase column coupled with UV and fluorescence detector was adopted based on these two publications. In the preliminary study, UV 350 nm and fluorescence with excitation wavelength at 340 nm and emission wavelength at 430 nm were selected. A Hibar Lichrosorb 5 μm RP-18 column kept at 60°C and a mobile phase of methanol: water (43/57, v/v) containing 0.005 M triethyl amine, 0.0025 M sodium acetate, and 0.0025 M acetic acid (pH 4.5) pumped at 1.5 ml/min flow rate were performed. In this HPLC system, FWA standards were detected by UV detector prior to fluorescence detector resulting in discrepancy in retention times of peaks detected by these two detectors. Furthermore, the sensitivity of detection on FB257 was low. The column used in this system was poor in FWA selectivity. As shown in Figure 3, a satisfactory resolution between FB90 and FB266 was not achieved and the broad peaks on FB351, FB121, and FB71 were observed. Another HPLC system, in which a Prodegy 5 μm ODS(2) column and a mobile phase of acetonitrile: 0.01 M sodium dihydrogen phosphate (35/65, v/v) were carried out, was capable of

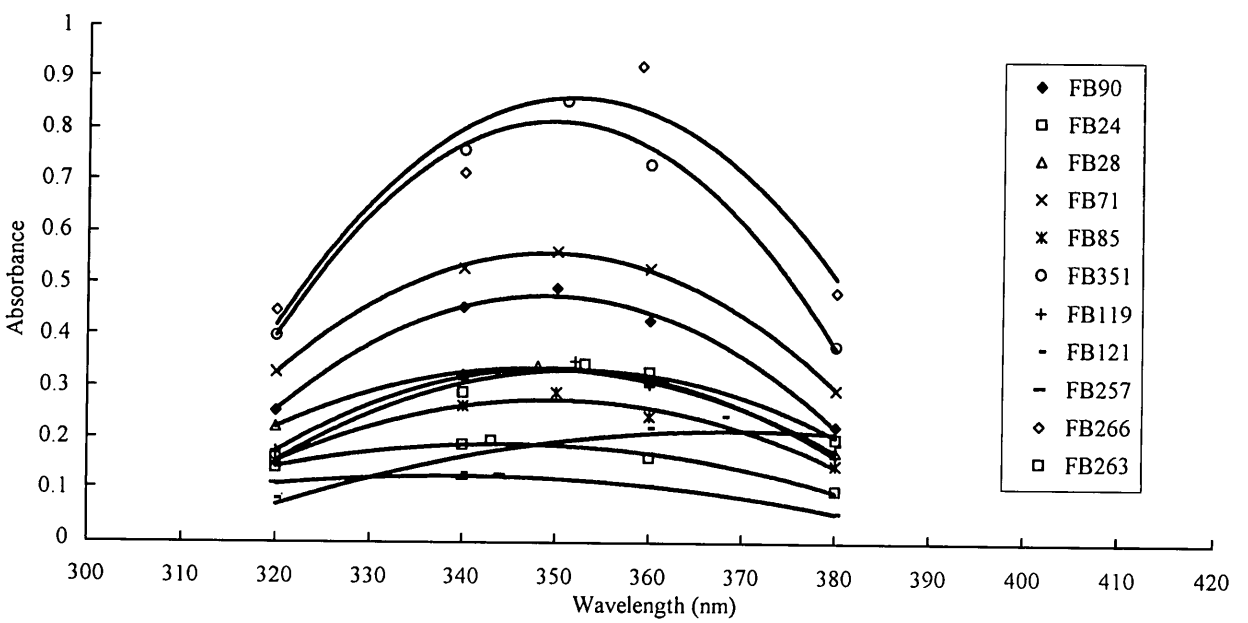

Figure 1. UV absorbance spectra of fluorescent whitening agents.

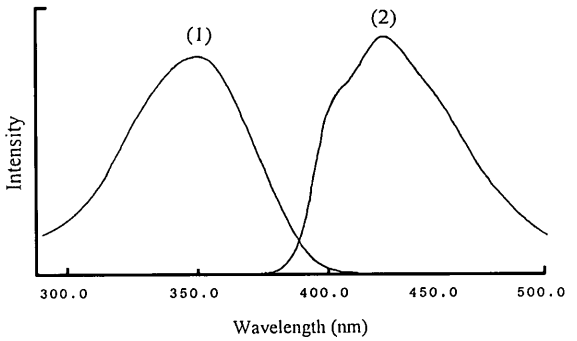

Figure 2. Fluorescence spectra of FB90 in mobile phase.
(1) Excitation spectrum at 430 nm emission, (2) Emission spectrum at 340 nm excitation.

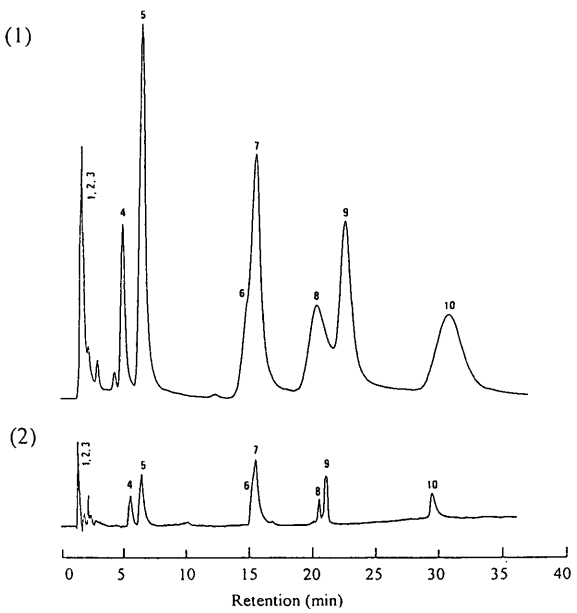

Figure 3. Chromatograms of a standard mixture of fluorescent whitening agents determined by HPLC using Lichrosorb RP-18 column.
(1) Detection with excitation 340 nm and emission 430 nm, (2) Detection with UV 350 nm.
HPLC conditions: column, Lichrosorb RP-18 5 μm (4.6 mm × 250 mm); mobile phase, methanol: H_2O (43:57) containing 0.005 M triethylammonium chloride, 0.0025 M sodium acetate and 0.0025 M acetic acid; flow rate, 1.5 ml/min; temperature 60°C; detector, (1) fluorescence detector (λex: 340 nm, λem: 430 nm), (2) UV detector 350 nm; injection volume, 10 μl.
Peaks: 1, FB24; 2, FB119; 3, FB263; 4, FB85; 5, FB28; 6, FB90; 7, FB266; 8, FB351; 9, FB121; 10, FB71.

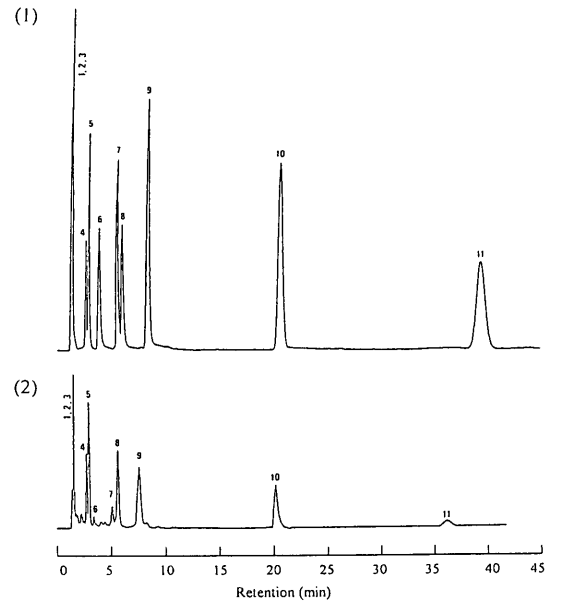

Figure 4. Chromatograms of a standard mixture of fluorescent whitening agents determined by HPLC using Prodegy 5 μm ODS (2) column.
(1) Detection with excitation 340 nm and emission 430 nm, (2) Detection with UV 350 nm.
HPLC conditions: column, Prodegy 5 μm ODS (2) (4.6 mm × 150 mm); mobile phase, acetonitrile: 0.01 M NaH_2PO_4 (35:65); flow rate, 1.0 ml/min; detector, (1) fluorescence detector (λex: 340 nm, λem: 430 nm), (2) UV detector 350 nm; injection volume, 10 μl.
Peaks: 1, FB24; 2, FB119; 3, FB263; 4, FB85; 5, FB28; 6, FB257; 7, FB90; 8, FB351; 9, FB71; 10, FB121; 11, FB266.

yielding a satisfactory resolution and detection sensitivity on these 11 FWAs as demonstrated in Figure 4. Figure 5 shows the effect of acetonitrile on capacity factors (K') of FWAs. K' value decreased with increasing acetonitrile concentration. A tailing effect on peaks was observed when 30% acetonitrile was used (data not shown). Figure 6 demonstrates the effect of sodium dihydrogen phosphate concentration over the ranges of 0.005~0.05 M on the K'. Results showed that K' decreased when the concentrations of sodium dihydrogen phosphate were lower than 0.01M. While in higher concentrations (> 0.01M), the orders of some peaks in retention time changed, resulting in a confusion in peak identification.

Therefore, a concentration of 0.01M sodium dihydrogen phosphate was selected in this study because it was capable of giving an optimum K'.

(III) *Solubility and Stability of FWAs*

Acetonitrile: water (30/70, v/v) solution showed best solubility of FWAs, followed by methanol, water, and acetonitrile. The solubilities in above solvents were 96, 85, 74, and 30 mg/ml, respectively. Among FWAs, FB266 gave the low-

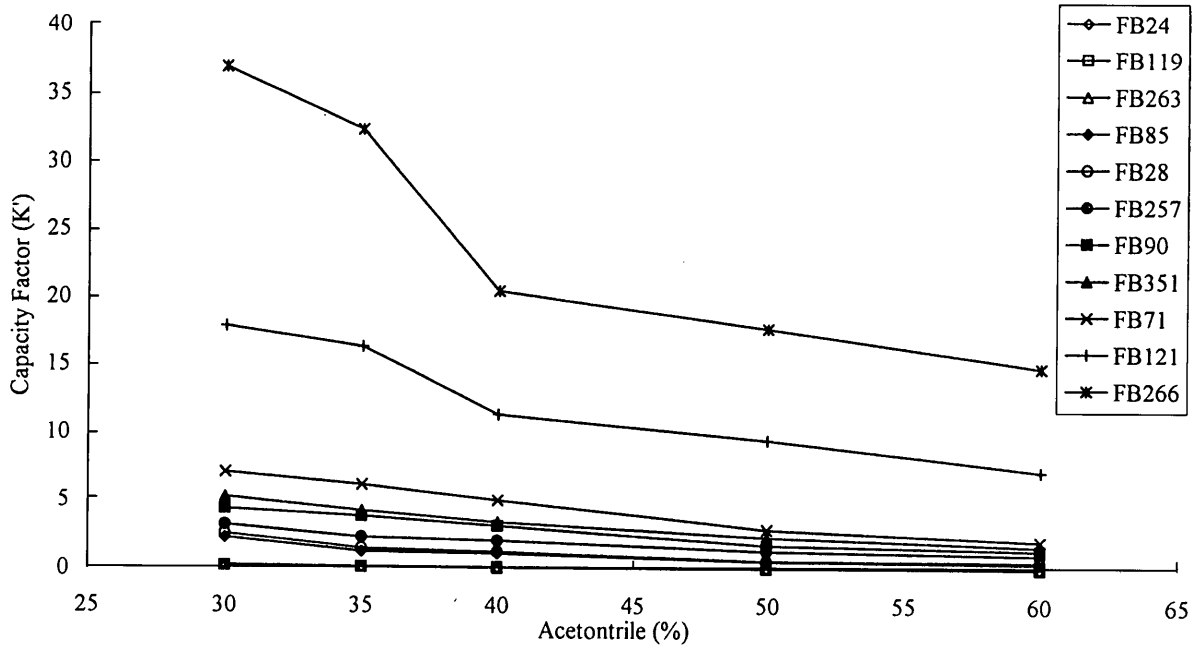

Figure 5. Effect of acetonitrile concentration (in mobile phase) on capacity factor (K') of fluorescent whitening agents.

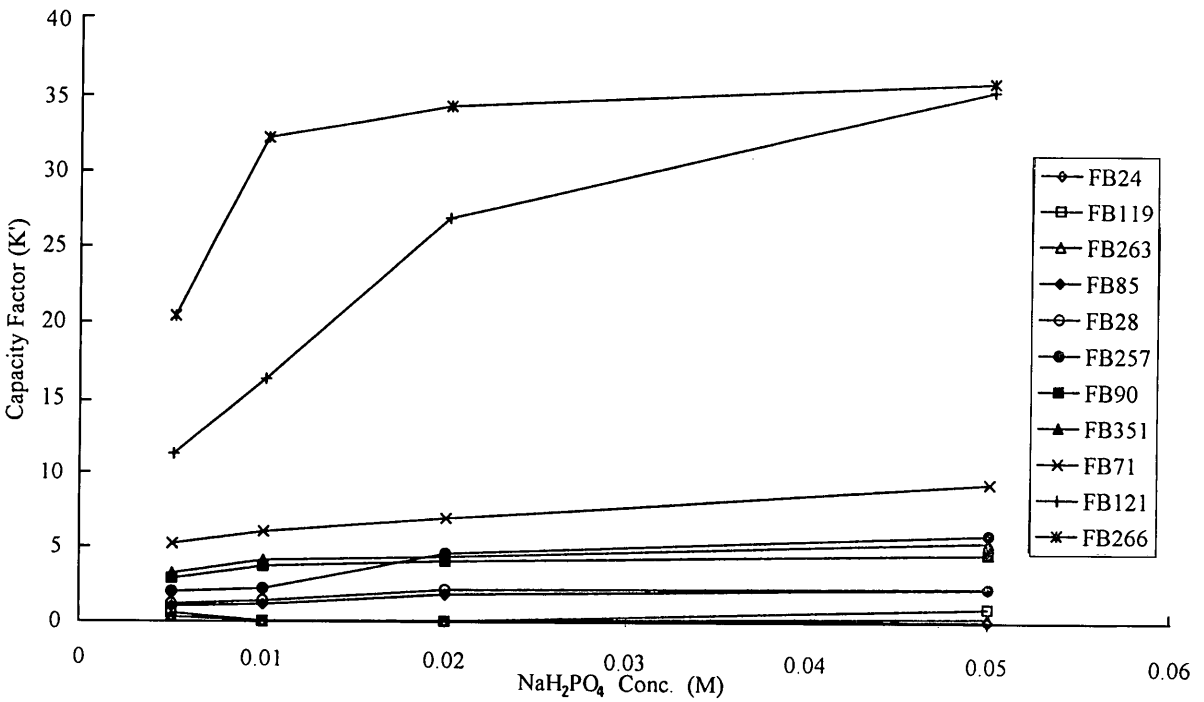

Figure 6. Effect of NaH_2PO_4 concentration on capacity factor (K') of fluorescent whitening agents.

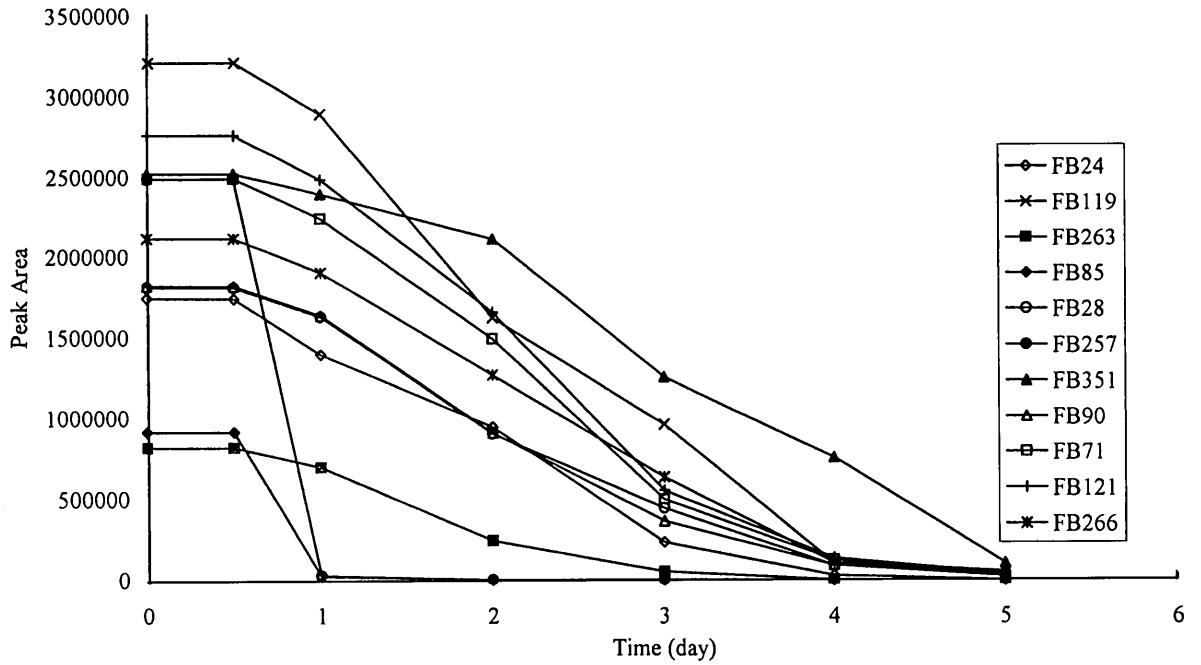

Figure 7. Stability of fluorescent whitening agents exposed to sunlight.

Table 4. Linear response of fluorescent whitening agents determined by HPLC

FWA	Slope	Intercept	r
FB24	1712436	27684	0.9995
FB28	3613161	34656	0.9983
FB71	4913520	62211	0.9990
FB85	894576	15444	0.9995
FB90	1788919	38147	0.9992
FB119	3183552	14965	0.9997
FB121	2738073	17343	0.9998
FB257	2450804	24949	0.9994
FB263	804820	18906	0.9994
FB266	2618	1024	0.9996
FB351	52152403	7117	0.9991

est solubility. HPLC detectable level for FB266 was higher than 10 ppm due to low solubility of this FWA.

In terms of stability, fluorescence intensity of FWAs decreases with the exposure time under sunlight due to a tautomerism from *trans* to *cis* form, an isomer with lower fluorescence intensity[13, 15]. The light effect is more pronounced on coumarins[1] and triazin-amino stilbenes. In this study, stability of these 11 FWAs was tested in the same solvents as solubility study. Stability of these FWAs under sunlight exposure is demonstrated in Figure 7. Results showed FWAs were poor in stability, especially FB85 and FB257, which reduced down to 2% after exposure to sunlight for only one day. Our study showed that standard solution can be stock for one week when kept in brown bottle at refrigerated temperature.

III. *Standard Curve Plotting*

Table 4 shows the linear response of FWAs over the range of 0.01~1 μg/ml for FB24,FB28, FB85, FB90, FB119, FB121, FB257, and FB263, of 0.005~0.5 μg/ml for FB71, of 0.001~0.1 μg/ml for FB351, and of 25~800 μg/ml for FB266. A satisfactory linearity with regression coefficience(r) higher than 0.99 for all FWAs was achieved.

IV. *Detection Limit of Instrument*

The limits of detection (S/N ≥ 3) for HPLC by fluorescence detector to FWAs with 10 μl injection were 0.01 ppm for FB24, FB85, and FB263, 0.005 ppm for FB28, FB90, FB119, FB121, and FB257, 0.002 ppm for FB71, 0.0005 ppm for FB351, and 10 ppm for FB266.

Our study showed that TLC could only be used to determine the existence of FWA in samples. Further identification of FWAs requires HPLC. HPLC with UV detection was not capable of revealing all tested FWAs compared to HPLC with fluorescence detection. The satisfying analytical results were obtainable when fluorescence detector coupled with a Prodegy 5 μm ODS(2) column and a mobile phase of acetonitrile: 0.01 M sodium dihydrogen phosphate (35/65, v/v) were used.

V. *Investigation of FWA Residues in Commercial Paper Containers*

The method developed in this study could efficiently identify the FWAs in samples. Investigation of FWAs in commercial products were also carried out. No FWA residues were found in 50 collected samples including paper plates, paper cups, and meal boxes. A periodical or sporadic inspection of commercial containers is strongly suggested in order to prevent FWAs from being illegal used although there was no FWA found in this investigation. The current HPLC method can still be used for the identification of other FWAs, which are not analyzed in this work. The extraction method needs further refinement in order to accurately quantify FWAs in samples.

ACKNKOWLEDGMENT

We thank Dr. C.W. Chen for his translation work.

REFERENCES

1. Chiu,Yung-Liang 1989. Stain chemistry. pp. 185-187. Shyu-Shih Foundation. Taipei, Taiwan.
2. Gloxhuber, C. and Bloching, H. 1978. Toxicologic properties of fluorescent whitening agents. Clinical Toxicol. 13: 171-203.
3. Pharmacentical Society of Japan. 1990. Test of paper, processed paper and cellophane containers. In "Standard Methods of Analysis for Hygienic Chemists--with Commentary". pp. 581-588. Tokyo, Japan.
4. Committee of Fluorescent Whitening Agents. 1976. Fluorescent whitening agents. In "Associ-ation of Industry of Fluorescent Whitening Agents Products". Tokyo, Japan.
5. Ministry of Economic Affairs. National Bureau of Standards. 1975. Detergents for vegetable, fruit and kitchen utensils. CNS 3800 S1085.
6. Department of Health, Executive Yuan. 1988. Method of Test for Fluorescent Whitening Agent in Food--Test of Diaminostilbene and Bis-styrylbiphenyl Derivatives. Ordinance No. 762034.
7. Ito, Y., Tonogai, Y., Toyoda, M., Suzuki, S.O. and Iwaida, M. 1980. Detection of fluorescent dye in boiled, dried larval fish. J. Food Hyg. Soc. Japan 21: 435-441.
8. Ganz, L. R., Liebert, C., Schulze, J. and Stensby, P. S. 1975. Removal of detergent flurescent whitening agents from wastewater. J. Water Pollut. Control Fed. 47: 2384-2849.
9. Lepri, L., Desideri, P. G. and Coas, W. 1985. High performance thin-layer chromatography of fluorescent whitening agents and their identification in detergents. J. Chromatogr. 322: 363-370.
10. Abe, A. and Yoshimi, H. 1979. Qualitative analysis of fluorescent whitening agents in river water. Water Research 13: 1111-1112.
11. Uchiyama, M. 1978. Separation and determination of fluorescent whitening agent and alkyl benzenesulfonate in water. Water Research 13: 847-853.
12. Micali, G., Curro, P. and Calabro, G. 1984. High-performance liquid chromatographic separation and determination of fluorescent whitening agents in detergents. Analyst 109: 155-158.
13. Tsuji, K., Naito, S. and Nakazawa, H. 1988. Determination of fluorescent whitening agents in river waters with high performance liquid chromatography. Hyg. Chem. 34: 501-507.
14. Kao, Y. M. and Chang, P. C. 1992. Separation and identification of fluorescent whitening agents in food. Annual Scientific Report of

National Laboratories of Foods and Drugs. Department of Health, Executive Yuan. 10: 74-83.
15. Pogier, T., Field, J. A., Field, T.M. and Giger, W. 1996. Occurrence of fluorescent whitening agents in sewage and river determined by solid-phase extraction and high-performance liquid chromatography. Environ. Sci. Technol.

紙製容器(餐盤、紙杯及便當盒)中螢光增白劑檢驗方法之探討

饒麥玲* 廖梅英 黃春子 鄭秋真 周薰修

藥物食品檢驗局食品化學組
台北市南港區昆陽街161-2號

摘 要

本研究利用高效液相層析法(high performance liquid chromatography，HPLC)分析紙製容器(餐盤、紙杯及便當盒)中之螢光增白劑(fluorescent whitening agents)。經依據衛生署公告第762034號之螢光增白劑檢驗方法，將紗布浸泡後，以二甲醯胺萃取，經減壓濃縮至乾，轉溶後再以HPLC分離鑑定。發現螢光增白劑可以下列之HPLC分析條件加以分離鑑定，即以Prodigy 5 μm ODS (2)爲分離管，乙腈:0.01 M磷酸二氫鈉(35:65，v/v)爲移動相，以螢光檢測器於激發波長340 nm及放射波長430 nm檢測。其次，以上述條件，分析五十件市售紙製容器，經確認結果均未檢出螢光增白劑。

關鍵詞：高效液相層析，螢光增白劑，紙製容器。