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Determination of Fluorescent Whitening Agents in Paper Materials by Ion-Pair Reversed-Phase High-Performance Liquid Chromatography

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A simple method was developed for the analysis of seven stilbene-type fluorescent whitening agents (FWAs) in paper materials by ion-pair reversed-phase high-performance liquid chromatography with fluorescence detection. These stilbene-type FWAs included two disulfonate, two tetrasulfonate, and three hexasulfonate compounds. After optimization of chromatographic conditions, the FWAs were satisfactorily separated using a reversed-phase column (RP-18) with the following isocratic mobile phase: methanol-water (60:40) containing 17.5 mM TBABr and 10 mM citrate buffer (pH = 7.0). The calibration plot was linear in the range from 5 to 500 ng/mL for two disulfo-FWAs and from 1 to 500 ng/mL for the other five FWAs. Precision levels of the calibration curve as indicated by RSD of response factors were 1.2 and 8.1%. Limits of quantitation (LOQ) ranged from 1.2 to 11 ng/mL.

Key Words : Fluorescent whitening agents, Ion-pair chromatography, Paper materials

Introduction

Fluorescent whitening agents (FWAs) are widely used in papers, textiles, and household detergents to enhance the whiteness characteristics of products. FWAs used in paper and board are mainly based on diaminostilbene derivatives due to their fastness properties, and they are traditionally divided into disulfo-, tetrasulfo-, or hexasulfo-FWAs depending on the number of sulfonate groups present. Disulfo-FWAs are used in the wet end process to increase the whiteness of base paper as they strongly bond with paper fibers. Tetrasulfo-FWAs, which also have sufficient affinity for fibers, are widely used in the wet end, sizing, and coating processes. Although hexasulfo-FWAs have low affinity for fibers, they increase whiteness better than other FWAs. Accordingly, hexasulfo-FWAs are used in sizing and coating processes to attain high whiteness levels.[1,2] Although the toxicity of FWAs used in paper is low, some of the chemicals used as FWAs may be harmful to human health. Therefore, FWAs are not allowed in paper and board that contact food due to potential contamination.[3]

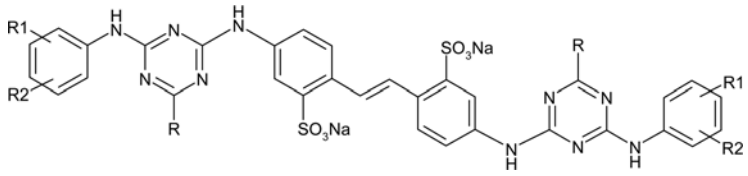
Conventionally, the content of FWAs in paper has been determined through a spectrophotometric method.[4,5] In this method, the total amount of FWAs in paper can be only measured. For risk assessment of FWAs, quantitative analyses of individual FWAs are necessary to determine their various toxic effects. A number of methods for the determination of individual FWAs have been applied, including thin-layer chromatography (TLC),[6] capillary zone electrophoresis (CZE),[7] high-performance liquid chromatography (HPLC),[8-10] and liquid chromatography-mass spectrometry (LC-MS).[11,12] Although a number of reports have demonstrated the use of FWAs in paper and board, they have mainly focused on disulfo-FWAs[6,10,11] or anilinosulfonic acid derivatives of 4,4'-bis[[4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulfonate salt.[7-9,12] However, the analytical method for various FWAs used in paper and board has not been reported yet. Thus, it is necessary to develop an analytical method that can determine various FWAs.

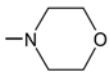
In this study, we developed a simple and reliable method to simultaneously determine seven selected FWAs used in paper making industry. The FWAs were chosen according to their practical importance in the paper industry. We applied hot-water extraction as well as an isocratic ion-pair chromatographic method with fluorescence detection. The chromatographic conditions were optimized through detailed study of the effects of the mobile phase components on the retention behavior of the FWAs. Recovery of hot-water extract and analytical performance were evaluated, and the FWAs in take-out pizza box samples were determined.

Experimental

Chemicals and Reagents. Tetrabutylammonium bromide (TBABr), citric acid monohydrate, and sodium citrate tribasic dihydrate were purchased from Junsei Chemical Co. (Tokyo, Japan). All chemicals were guaranteed as reagent grade and were used without further purification. Methanol (MeOH) of HPLC grade was obtained from J. T. Baker (Philipsburg, NJ, USA). Deionized water was purified through a purification system that gives conductivity above 18 MΩ/cm. Seven industrial grade FWAs commonly used by Korean paper and paperboard manufacturers were obtained from KISCO (Seoul, Korea). The following FWAs were used: (1) Disulfo-FWA (D hereafter; Color Index FB 28; disodium

Table 1. Structures of seven FWAs



Type	FWA	R	R1 (Position)	R2 (Position)	CAS Registry no.	Color index
Disulfo	D	$-N(CH_2CH_2OH)_2$	H	H	4193-55-9	FB 28
	D-M	$-N(CH_2CH_2OH)(CH_2CH_2CONH_2)$	H	H	68444-86-0	FB 230
Tetrasulfo	T	$-N(CH_2CH_2OH)_2$	$-SO_3Na$ (4)	H	16470-24-9	FB 220
	T-M	$-N(CH_2CHCH_3)_2$ (OH)	$-SO_3Na$ (4)	H	99549-42-5	CI 263
Hexasulfo	H	$-N(CH_2CH_2OH)_2$	$-SO_3Na$ (2)	$-SO_3Na$ (5)	76482-78-5	FB 264
	H-M1		$-SO_3Na$ (2)	$-SO_3Na$ (5)	55585-28-9	FB 353
	H-M2	$-N(C_2H_5)_2$	$-SO_3Na$ (2)	$-SO_3Na$ (5)	83512-97-4	FB 357

4,4'-bis[[4-[bis(2-hydroxyethyl)amino]-6-anilino-1,3,5-triazin-2-yl]amino] stilbene-2,2'-disulfonate salt); (2) Modified disulfo-FWA (D-M; Color Index of disulfonate compound FB 230); (3) Tetrasulfo-FWA (T; Color Index FB 220; tetrasodium 4,4'-bis[[4-[bis(2-hydroxyethyl)amino]-6-(4-sulfonateanilino)-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulfonate salt); (4) Modified tetrasulfo-FWA (T-M; Color Index of tetrasulfonate compound C.I. 263); (5) Hexasulfo-FWA (H; Color Index FB 264; hexasodium 4,4'-bis[[4-[bis(2-hydroxyethyl)amino]-6-(2,5-disulfonate anilino)-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulfonate); (6) Modified hexasulfo-FWA (H-M1; Color Index FB 353); (7) Modified hexasulfo-FWA (H-M2; Color Index FB 357). The structures of these seven FWAs are shown in Table 1.

Individual stock solutions (1,000 g/mL) were prepared by dissolving the FWAs in deionized water. Standard solutions were prepared by dilution of these stock solutions with an appropriate volume of deionized water. All stock solutions and standard solutions were stored in a refrigerator (4 °C, in the dark) to prevent light-induced conversion of the *trans*-isomers of the FWAs to *cis*-isomers.

Paper Sample Preparation. Paper samples were single-wall corrugated board from take-out pizza containers. For this, various pizza containers were obtained from nationwide pizza makers. Procedures for the preparation of hot water extract were performed as reported elsewhere, except with slight modifications.[13] Briefly, a paper sample (10 g) was cut into 1-2 cm^2 pieces and placed into a bottle (500 mL) for hot-water extraction. Hot deionized water (200 mL at 80 °C) was then added and the bottle maintained at 80 °C for 2 h in the dark. The resulting aqueous extract was allowed to cool, followed by filtration through a 0.45 mm membrane filter (Whatman). Extracts were kept in a refrigerator until analyzed by HPLC.

Recovery experiments were performed using the spiked paper samples. For this, a known amount (10 μg/g) of FWA standard methanolic solution was evenly distributed on the paper sample using a glass syringe. The spiked samples were stored in a convection oven at 70 °C for 1 h and then allowed to equilibrate at room temperature for at least 2 h in the dark.

HPLC Analysis. All chromatographic experiments were carried out on a Hewlett-Packard 1100 chromatographic system (Agilent Technologies, Waldbronn, Germany) equipped with an in-line degasser (G1322A), quaternary pump (P580A), auto sampler (GINA50), thermostatted column compartment (G1316A), and fluorescence detector (G1315A). Separations were performed on an Optimapak C18 column with dimensions of 150 mm × 4.6 mm I.D. (RS tech Co., Daejeon, Korea). The column had an average particle size of 5 μm and an average pore size of 100 Å. The column was kept at 35 °C in a column oven. The mobile phase was delivered at a flow-rate of 1.0 mL/min with isocratic elution. The injection volume was 10 μL, and fluorescence detection was operated at an excitation wavelength of 360 nm and emission wavelength of 425 nm. Analytical performance was evaluated, and the FWAs in take-out pizza box samples were determined.

Results and Discussion

Optimization of Chromatographic Conditions. As all seven FWAs are sulfonate sodium salts, each is a strong acid

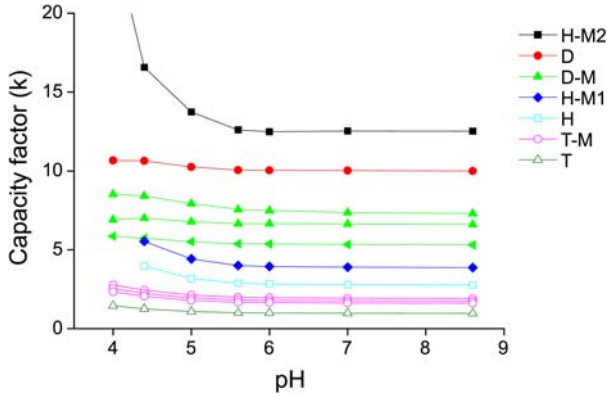


Figure 1. Effect of eluent pH on the retention and separation of FWAs. Mobile phases: 60/40 of MeOH-10 mM citrate buffer containing 17.5 mM TBABr.

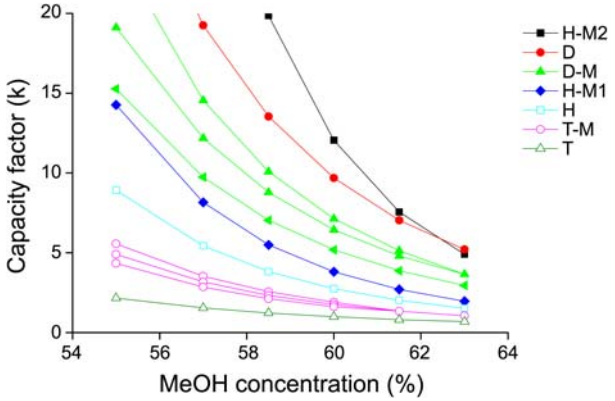


Figure 2. Effect of MeOH concentration on the retention and separation of FWAs. Mobile phases: MeOH-10 mM citrate buffer (pH 7.0) containing 17.5 mM TBABr.

with a pK_a value below −1. Hence, the FWAs could be separated using a mobile phase with a positively charged ion pair reagent that forms a neutral ion-pair with the anionic FWAs in the neutral pH range. To optimize the retention and separation of the ionic FWAs, several experimental parameters should be adjusted. In this work, the selected parameters were the pH of the mobile phase, the concentrations of tetrabutylammonium bromide (TBA-Br) as an ion-pairing agent and citrate buffer, and the percentage of methanol as an organic modifier. Unless otherwise specified, the composition of the mobile phase was methanol-water (60:40) containing 17.5 mM TBABr and 10 mM citrate buffer (pH 7.0). The column temperature was kept at 35 °C in a column oven.

The pH of the mobile phase is a key factor in controlling the degree of ionization of the ionic analytes as well as the magnitude of interactions between the ion-paired analytes and stationary phase in ion-pair RP-LC.[14] The effect of pH on retention of the FWAs was investigated in the pH range from 4.0 to 8.6 (Figure 1). Retention of the seven FWAs was not affected by changes in the mobile phase pH, and separation was satisfactory in the pH range from 6.0-8.6. It was observed that the seven FWAs were negatively charged anions at higher pH values, thus forming neutral ion pairs with tetrabutylammonium positive ion, and remained on the C18 column as the stationary phase. On the other hand, in the pH range from 4.0 to 6.0, retention of all FWAs increased with decreasing mobile phase pH, and the peak shape of the FWAs was broad and asymmetric. It is believed that long retention time and peak asymmetry result from the presence of a quaternary ammonium cation at low pH.[15] Therefore, a mobile phase pH value of 7.0 was chosen not only for optimum separation but also for longer column life.

The concentration of organic modifier in the mobile phase strongly influences the retention of ionic analytes in ion-pair RP-LC.[16] Accordingly, the effect of the concentration of methanol as an organic modifier on retention of the FWAs was studied (Figure 2). Retention times of these seven FWAs that form ion pairs with tetrabutylammonium bromide drastically decreased as the methanol concentration of the mobile phase increased. Further, at lower concentrations of methanol (55-59%), it was possible to obtain satisfactory separation of the seven FWAs, but the retention times were too long (> 25 min). As the methanol concentration increased to higher than 61%, separation of the seven FWAs could not be achieved. Therefore, the methanol concentration of the mobile phase was selected to be 60%, thereby enabling satisfactory separation of the seven FWAs within a reasonable retention time.

The effect of the concentration of tetrabutylammonium bromide as an ion pair reagent on retention of the seven FWAs is shown in Figure 3. Retention behavior of these FWAs was dependent upon the number of sulfonate groups in the FWA molecules. Tetrasulfo-FWAs and hexasulfo-FWAs were not retained without the addition of tetrabutylammonium bromide as an ion pair reagent, whereas disulfo-FWAs were retained. In the absence of an ion pair reagent, retention of disulfo-FWAs indicated the occurrence of hydrophobic interactions with the stationary phase.[14] Retention of disulfo-FWAs rapidly increased with increasing concentration of tetrabutylammonium bromide to 15 mM, whereas it gradually increased from 15 to 25 mM. On the other hand, retention of hexasulfo-FWAs, especially that of H-M2 FWA,

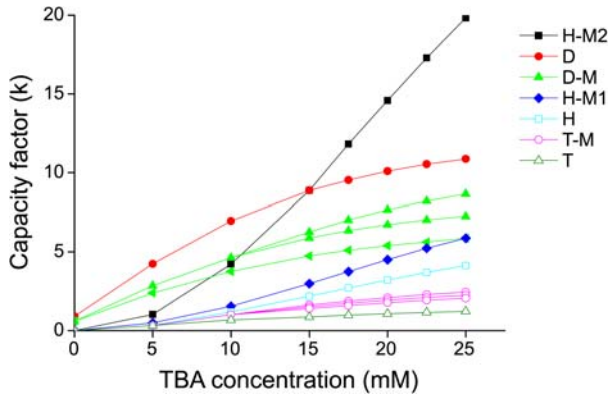


Figure 3. Effect of TBABr concentration on the retention and separation of FWAs. Mobile phases: 60/40 of MeOH-10 mM citrate buffer (pH 7.0) containing TBABr.

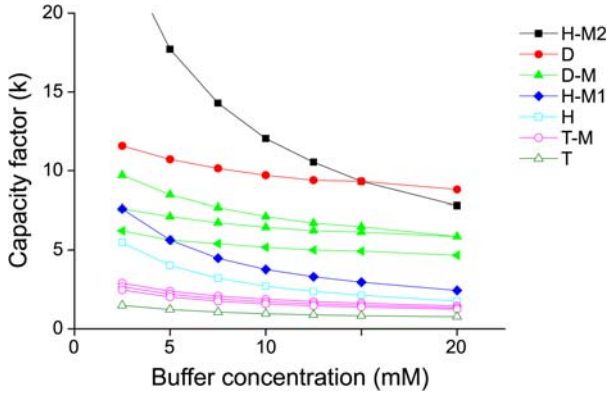


Figure 4. Effect of citrate buffer concentration on the retention and separation of FWAs. Mobile phases: 60/40 of MeOH-citrate buffer (pH 7.0) containing 17.5 mM TBABr.

continuously increased from 0 to 25 mM. It is believed that the presence of tetrabutylammonium bromide is needed more for hexasulfo-FWAs to pair all sulfonate groups in FWA molecules than for disulfo-FWAs.[17] Even so, the optimum concentration of tetrabutylammonium bromide was 17.5 mM for adequate separation and reasonable retention of these seven FWAs.

The effect of citrate buffer concentration on retention of the seven FWAs is shown in Figure 4. Retention times of the seven FWAs decreased with increasing buffer concentration, and retention behavior was dependent upon the number of sulfonate groups in the FWA molecules. Retention of hexasulfo-FWAs, especially that of H-M2 FWA, rapidly decreased with increasing citrate buffer concentration from 2.5 to 20 mM, whereas retention of disulfo-FWAs and tetrasulfo-FWAs slowly decreased. Alteration of the retention order between H-M2 and D FWAs was observed as the concentration of citrate buffer increased to higher than 15 mM. However, at a buffer concentration of 10 mM, high reproducibility of the retention times of the FWAs could be obtained due to an adequate buffering effect. Therefore, the concentration of citrate buffer in the mobile phase was selected to be 10 mM in order to achieve excellent separation and a reasonable retention time.

The obtained chromatogram (Figure 5) showed excellent separation between all FWAs. Thus, the proposed method is suitable for specific determination of these FWAs.

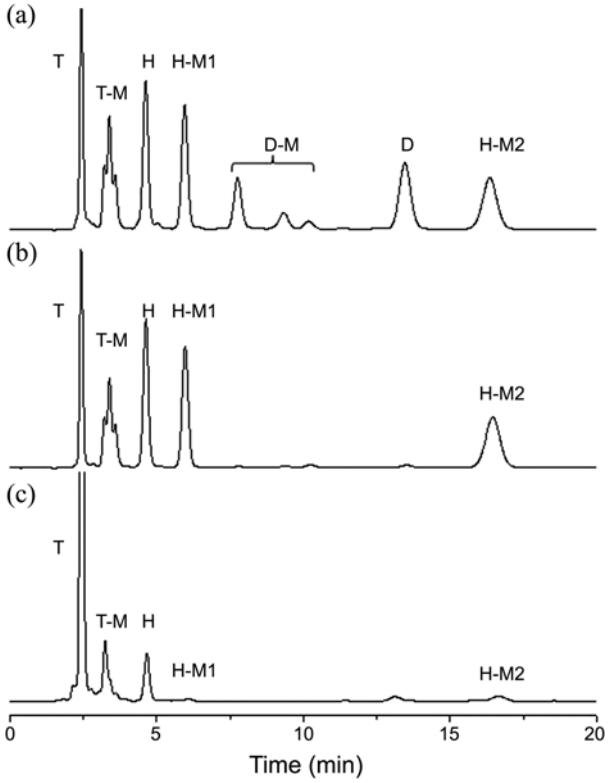


Figure 5. Chromatograms of (a) FWA standards, (b) FWA spiked paper extract and (c) commercial take-out pizza board extract.

Analytical Performance. The analytical characteristics of the method were investigated under the optimized chromatographic conditions in order to evaluate efficiency. Performance data, including linear response range, detection limit, and quantitation limit, are summarized in Table 2. Linearity of the seven FWAs was tested using the calibration curve (five-level) from 5 to 500 ng/mL for the two disulfo-FWAs and from 1 to 500 ng/mL for the other five FWAs. The RSD of response factors (RFs) ranged from 1.2-8.1%, and the correlation coefficients (r^2) exceeded 0.9999. A good linear relationship between the detector response and the corresponding sample concentration was observed from the calibration results. The limit of detection (LOD) of the analytes was calculated by the equation LOD = 3 × Sy/m (where m is the slope of the calibration graph and Sy is the residual standard deviation), and LOD values ranged from 0.3 to 3.3

Table 2. Linear-regression calibration results, LOD and LOQ for seven FWAs

FWA	t_R (min)	Range (ng/mL)	Equation	R^2	RF (RSD) (%)	LOD (ng/mL)	LOQ (ng/mL)
D	13.19	1-500	Y = 1.5972x – 0.8896	0.9999	3.3	1.4	4.8
D-M	7.62	5-500	Y = 0.7561x – 0.0762	0.9999	4.2	2.4	7.9
	9.17	5-500	Y = 0.2628x + 0.0127	0.9999	5.9	3.3	11.0
	9.97	5-500	Y = 0.1481x + 0.0121	0.9999	8.1	3.2	10.5
T	2.41	1-500	Y = 1.5878x – 0.0822	0.9999	1.2	0.4	1.2
T-M	3.35	1-500	Y = 1.5672x – 0.0239	0.9999	1.9	0.5	1.6
H	4.55	1-500	Y = 1.5760x – 0.0676	0.9999	2.6	0.6	1.9
H-M1	5.84	1-500	Y = 1.5319x – 0.1732	0.9999	2.7	0.3	1.0
H-M2	15.91	1-500	Y = 1.5350x – 0.1611	0.9999	1.8	0.5	1.7

Table 3. Recovery of seven FWAs from spiked papers

FWA	t_R (min)	Recovery (%)	RSD (%)
D	13.19	5.0	9.6
D-M	7.62	2.1	3.6
	9.17	2.3	7.5
	9.97	4.2	7.3
T	2.41	79.3	7.0
T-M	3.35	59.0	5.8
H	4.55	76.6	3.3
H-M1	5.84	67.0	1.9
H-M2	15.91	52.8	1.6

ng/mL. The limit of quantitation (LOQ) of the analytes was calculated by the equation LOQ = 3.3 × LOD, and LOQ values ranged from 1.2 to 11 ng/L.

Hot-water extraction has been extensively used to extract FWAs from paper and paperboard products for food contact use, but the reported recovery rates of the matrix-spiked samples vary among previous experiments. Recovery of FWAs from the spiked take-out pizza board was measured in order to examine the effectiveness and repeatability of hot-water extraction (Table 3). Recovery rates of the tetrasulfo-FWAs and hexasulfo-FWAs ranged from 52.8 to 79.3%, and the precision (RSD, n = 3) ranged from 1.6 to 7.0%. This value is comparable to the previous result by Santos *et al.*, where the precision level ranged from 3 to 8%.[8] On the other hand, recovery rates of disulfo-FWAs ranged from 2.1 to 5.0%, and the precision ranged from 3.6 to 9.6%. These results reflect relative affinity of each type of FWA to the cellulose fiber in paper.[2] The drying step in the recovery experiment simulate the papermaking process, enhancing the adhesion of FWAs to the cellulose fiber. Meanwhile, the experiment without drying resulted in nearly complete recovery (not shown here). Hot-water extraction is a useful method for the extraction of FWAs from paper materials for food contact use.[13]

Applications. Five commercial take-out pizza board samples were analyzed for evaluation of the proposed method. A typical ion-pair chromatogram obtained from the analysis of a take-out pizza board sample is shown in Figure 5. The peaks in the chromatogram were identified by comparison with the retention times of standard FWAs, and quantities were calculated by an external standard method. The amounts of the seven FWAs in board samples from different manufacturers are shown in Figure 6. Various FWAs were detected in the take-out pizza board samples. Specifically, T FWA was shown to be a major FWA as it was found in all five pizza board samples and measured in the concentration range from 40.9 to 59.7 μg/g. Hexasulfo-FWAs and T-M FWA were also detected, but disulfo-FWAs were not detected due to strong bonding with paper fibers. These results reveal that the ion-pair RP-LC method developed in this study is suitable for analyzing various FWAs in paper materials.

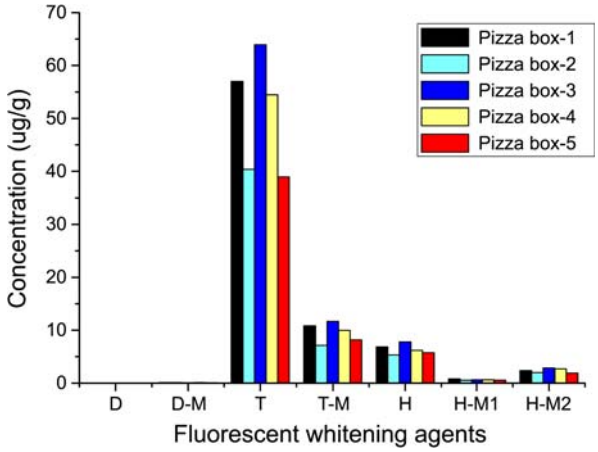


Figure 6. Contents of FWA found in commercial take-out pizza boards.

Conclusion

A reversed-phase ion-pair HPLC method was developed for the simultaneous determination of stilbene-type FWAs with different sulfonate groups. In the separation of these seven FWAs, the selection of pH range was the most important factor for the stable retention of FWAs, and the concentrations of methanol and TBA-Br were the two parameters that most strongly affected the retention times and separation of FWAs. The method developed herein is also applicable as a quality control method for FWA products and as an analytical method for FWAs in process water from paper mills. Although hot-water extraction is reasonable for the analysis of extractable FWAs in paper materials for food contact use, the extraction efficiency of the method is very low. Accordingly, further research is required on an extraction method for the determination of FWAs in paper and board materials.

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References

1. Roberts, J. C., Ed.; Paper Chemistry; Blackie Academic and Professional: London, 1992.
2. Kaschig, J. In *Industrial Dyes Chemistry, Properties, Applications*; Hunger, K., Ed.; WILEY-VCH: Weinheim, Germany, 2003; Chapter 7, p 614.
3. Code of Federal Regulations, Foods and Drugs, Title 21, Parts 170-199. "Office of the Federal Register National Archives and Records Administration". Washington, DC: U.S. Government Printing Office. Revised April 1, 2007.
4. Lee, J. Y.; Youn, H. J.; Lee, H. L. *Bioresources* **2012**, *7*, 315.
5. Liu, J.; Qin, Z.; Zuo, Y.; Liu, S. *Paper and Paper Making* **2011**, *3*, 57.
6. Jao, M.; Liao, M.; Huang, C.; Cheng, C.; Chou, S. *J. Food Drug Anal.* **1999**, *7*, 53.
7. Damant, A. P.; Castle, L. *J. Microcol. Sep*. **1999**, *11*, 259.
8. De los Santos, M.; Batlle, R.; Salafranca, J.; Nerín, C. *J. Chromatogr. A* **2005**, *1064*, 135.
9. Kaji, M.; Enomae, T.; Isogai, A. *Japan TAPPI J.* **2008**, *62*, 590.
10. Shu, W.; Ding, W. *J. Chin. Chem. Soc.* **2009**, *56*, 797.

11. Chen, H.; Ding, W. *J. Chromatogr. A* **2006**, *1108*, 202.
12. Shadkami, F.; Helleur, R.; Sithole, B. B. *J. Wood Chem. Tech.* **2011**, *31*, 42.
13. European Standard EN647, Paper and Board Intended to Come into Contact with Foodstuffs. Preparation of a Hot Water Extract; 1993.
14. Cecchi, T. *Crit. Rev. Anal. Chem.* **2008**, *38*, 161.
15. Wang, S.; Huang, S. *Electrophoresis* **2001**, *22*, 2222.
16. Cecchi, T.; Pucciarelli, F.; Passamonti, P. *Chromatographia* **2003**, *58*, 411.
17. Cecchi, T., Pucciarelli, F.; Passamonti, P. *J. Liq. Chromatogr. & Rel. Technol*. **2004**, *27*, 1.