Characterization of Brighteners in Detergents by High-Performance Liquid Chromatography/Mass Spectrometry/Ultraviolet/Fluorescence and Three-Dimensional High-Performance Liquid Chromatography

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ABSTRACT: A method has been developed for the identification of unknown brighteners in detergents by a high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/ultraviolet (UV)/fluorescence system with MS/MS capability. Ten brighteners from five groups—stilbene, biphenyl stilbene, pyrazoline, oxazole, and coumarin derivatives—were separated on a C_8 reverse-phase HPLC column. The UV and fluorescence detectors positively located the brightener peaks and differentiated between *trans* and *cis* isomers separated by the chromatographic system. Ion-spray (IS)/MS and IS/MS/MS spectra were then used to identify the structure of unknown brighteners in detergent products. An HPLC with a diode-array detector provided a quick identification check and accurate quantitative data for known brighteners, even on overlapping peaks. *JAOCS 72,* 827–833 (1995).

KEY WORDS: Biphenyl stilbene, brightener, detergent, fluorescence, fluorescence whitening agent, liquid chromatography, mass spectrometry, stilbene, *trans-cis* isomerization, ultraviolet.

Whiteness is a key indicator of cleanliness of laundry fabrics. However, laundry fabrics have a weak absorbance in the visible light spectrum around 400–450 nm, even after washing, and are perceived as yellow-colored by human eyes. Brighteners are organic compounds that are able to convert invisible ultraviolet (UV) light (290–400 nm) into visible blue light, which compensates for the yellow hue in laundry fabrics (1,2). Brighteners are added (0.01–0.6%) to almost all commercial detergents because of this reason. Currently, several hundred brighteners are available in the market and are classified into five groups, e.g., stilbene, biphenyl stilbene, pyrazoline, oxazole, and coumarin derivatives (1,2). They also are used in plastics, paper, pulp, and textile products.

Until 1980, brighteners were analyzed by thin-layer chromatography (TLC) as described by Mcpherson and Omelczenko (3), Theidel (4), and Anders (5). Since the development of high-performance liquid chromatography (HPLC), brighteners in detergents, river waters, and airborne dusts have been analyzed by HPLC with UV or fluorescence detectors. The reverse-phase HPLC method provides more precise and accurate data for the identification and quantitation of brighteners and is faster than TLC (3,6–11). At least 11 optical brighteners can be separated and quantitated by the HPLC method. However, it is impossible to identify unknown brighteners by the HPLC method without appropriate standards. Also, because several brighteners coelute with the HPLC method, more accurate identification approaches are needed. The research discussed in this paper focuses both on the identification of unknown brighteners in detergents and on obtaining more accurate quantitative information on known brighteners in detergents.

Recent progress on various types of mass spectrometry (MS) has contributed to the characterization of dyes (12–15) that are quite similar in structure to brighteners. In this research, ion-spray MS (IS/MS) was used to identify unknown brighteners in detergents. Brighteners were first separated by HPLC and subsequently identified based on their MS or MS/MS spectra. IS/MS is a soft-ionization system that can be easily coupled to an HPLC (16–20). The optimization study of conditions for the HPLC/MS/UV/fluorescence system and its application to a commercial granular detergent containing brighteners will be discussed.

The value of an HPLC system with a diode-array detector is also demonstrated for use on a daily basis to provide accurate quantitative analysis of known brighteners in detergent samples. This HPLC approach verifies the identity of known brighteners even on brighteners that coelute from the HPLC column.

EXPERIMENTAL PROCEDURES

Chemicals. Six stilbene derivatives and one biphenyl stilbene derivative were obtained from Ciba Geigy (Basel, Switzerland), one pyrazoline derivative was obtained from Mobay (North Charleston, SC), one oxazole derivative was obtained from Sumitomo Kagaku (Osaka, Japan), and one coumarin derivative was obtained from Aldrich Chemical Company (Milwaukee, WI). The structures of these compounds are shown in Figure 1. HPLC-grade methanol, acetonitrile, water,

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anhydrous disodium hydrogen phosphate, and ammonium acetate used for this research were purchased from Wako Junyaku Kogyo Co. Ltd. (Osaka, Japan).

Equipment. An HPLC/MS coupled with UV and fluorescence detectors was used for the characterization of brighteners. The eluent from the HPLC was split into two streams, one for the IS/MS, and the other for the UV and fluorescence detectors. The UV and fluorescence detectors were coupled in series.

The HPLC instrument was a Waters (Milford, MA) LC 625 system with a 486 UV detector and a 470 fluorescence detector. A degassor, model DGU 4A, from Shimadzu (Kyoto, Japan) and an injector, model 7125, from Rheodyne (Cotati, CA) with a 20-μL loop were attached to the system. The HPLC column was a C8 μ-Bonda Sphere (15 cm × 3.9 mm i.d.) from Waters Japan (Osaka, Japan).

The mobile phase was a mixture of acetonitrile (A) and 5 mM ammonium acetate in water (B). The initial ratio of solvents (A/B) was 20:80, and the final ratio was 100:0. A linear gradient was started immediately after injection, completed in 30 min, and a final hold time of 20 min was used to clean the column. Flow rate for the HPLC was 0.8 mL/min. Through the splitter, 10 μL/min of the HPLC eluent went to IS/MS, and the rest went to the UV and fluorescence detectors.

The wavelength of the UV detector was set at 340 nm for the selective detection of brighteners. The excitation wavelength of the fluorescence detector was 340 nm, and the fluorescence emission wavelength was 390 nm. The outlet of the UV detector was connected in series to the fluorescence detector with a poly ether ether ketone (i.d. = 100 μm) line.

An HPLC with a diode-array detector, model 1090M from Hewlett Packard (Palo Alto, CA), was used for routine verification of identity and quantitation of known brightener compounds in various products. The scan range for the detector was from 200 to 600 nm. The HPLC column, used for routine quantitation, was the same as that used for the HPLC/MS work. The mobile phase was acetonitrile/water (25:75) with 0.3% disodium hydrogen phosphate. The flow rate was 0.8 mL/min with isocratic elution of the brightener compounds.

An API-III interface from PE-Sciex (Toronto, Canada) was used between the HPLC and the Sciex MS/MS system. The orifice voltage for the API interface was –90V for stilbene and biphenyl stilbene derivatives and +90V for pyrazoline, oxazole, and coumarin derivatives. The collision gas used to generate daughter ions for MS/MS analysis was Argon (380×10^{12} molecules/cm^2). The collision energy given was 28 eV.

Standards and sample preparation. Before analysis with the LC/MS/UV/fluorescence system, the ten brightener standards, shown in Figure 1, were dissolved in acetonitrile/water (50:50) and prepared as a 1000 ppm (wt/vol) stock solution. The stock solution was then diluted to about 50 ppm (wt/vol) with acetonitrile/water containing 0.10M ammonium acetate (50:50). Five grams of granular detergent samples were ground and then slurried in 100 mL of acetonitrile/water (50:50) to dissolve the brightener compounds. The slurry was then filtered with a 0.45 μm Millex-HV filter from Millipore Corporation (Bedford, MA). Finally, 0.5 mL of the acetonitrile/water solution was diluted with 0.5 mL of water containing 0.10M ammonium acetate. This solution was used for all HPLC/MS analyses on detergent products.

Before analysis by the HPLC with a diode-array detector, the 1000-ppm stock solution was diluted to 3–15 ppm with acetonitrile/water (50:50) containing 0.3% disodium hydrogen phosphate. Three grams of ground granular detergent were slurried in 100 mL of acetonitrile/water (50:50) containing 0.3% disodium hydrogen phosphate and filtered. Twenty microliters were injected to the HPLC with an auto-sampler connected to the HPLC.

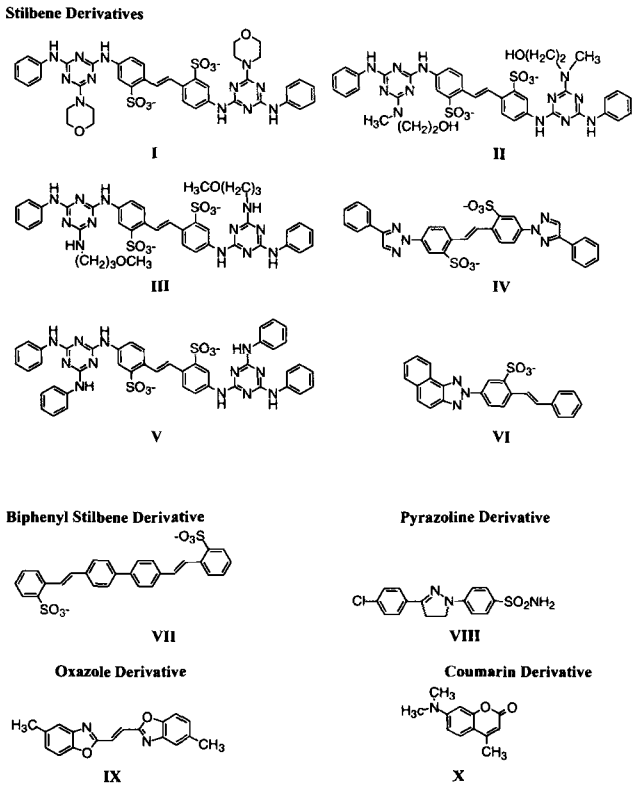

FIG. 1. Structure of compounds tested: **I.** *bis* (anilino-morpholino-triazinylamino) stilbene disulfonate, **II.** *bis* (anilino-hydroxyethylmethylamino-triazinylamino) stilbene disulfonate, **III.** *bis* (anilino-methoxypropylamino-triazinylamino) stilbene disulfonate, **IV.** *bis* (phenyl-triazolyl) stilbene disulfonate, **V.** *bis* (di-anilino-triazinyl-amino) stilbene disulfonate, **VI.** naphtho-triazolyl stilbene sulfonate, **VII.** *bis* (styrylsulfonate) biphenyl, **VIII.** chlorophenyl-pyrazolinyl benzenesulfonamide, **IX.** *bis* (methylbenzoxazolyl) ethylene, **X.** dimethylaminomethyl coumarin.

RESULTS AND DISCUSSION

Study on LC/MS/UV/fluorescence conditions. Prior to the LC/MS optimization study, IS/MS conditions and solvents for brighteners were studied by infusion/MS. Figure 2 demon-

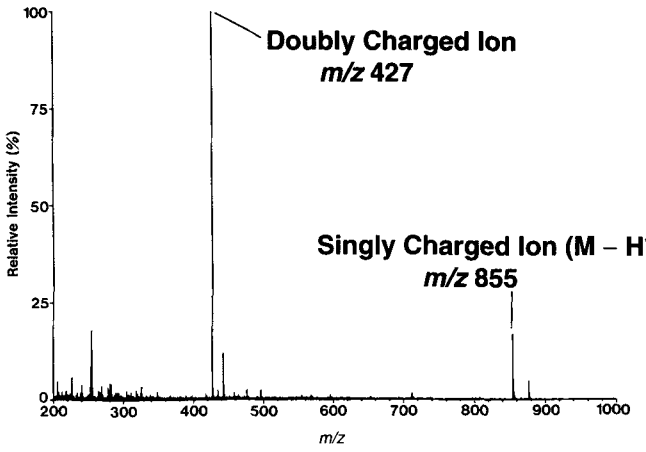

FIG. 2. Ion-spray/mass spectrometry spectrum for brightener II (stilbene derivative) in the negative-ion mode.

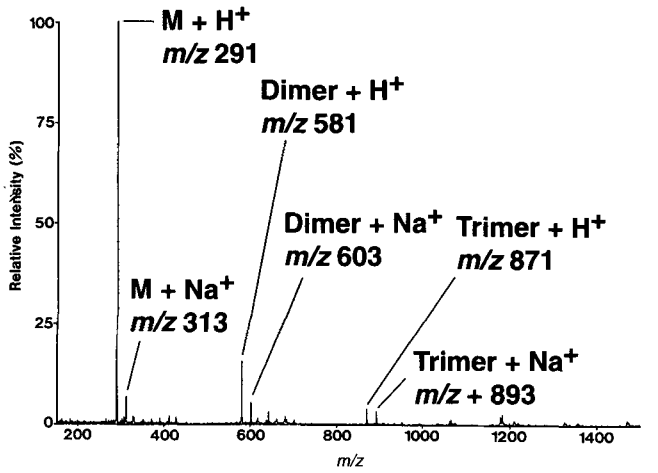

FIG. 3. Ion-spray/mass spectrometry spectrum for brightener IX (oxazole derivative) in the positive-ion mode.

strates that stilbene derivatives are effectively ionized in the negative-ion mode of the mass spectrometer. Biphenyl stilbene derivatives are also effectively ionized in the negative-ion mode. Multiple-charged ions are generated, depending on the number of sulfonates in the molecule. Figure 3 demonstrates that oxazole derivatives are ionized in the positive-ion mode and generate protonated ions, sodium adduct ions, and protonated or sodium adduct dimers and trimers. Ionization of pyrazoline and coumarin derivatives occurs in the same manner as oxazole.

Table 1 lists the results of the optimization study on matrices (solvents) for analyzing brighteners. There is a tendency for the ion intensity of brighteners to be lower in a matrix of acetonitrile/water (50:50) compared to their intensity in acetonitrile that contains a small amount of water (5%). This is caused by an increase in the surface tension of the solvent droplets at the ion source as the water concentration increases (21,22). The increased surface tension interferes with the ion-evaporation process of the brightener solutions. However, the presence of ammonium acetate increased the efficiency of the ionization of brighteners. The presence of the ionic buffer increases the conductivity of the solution and promotes the ionization of compounds in both liquid and gas phases (23,24). Ion intensities were further optimized by adjusting the solvent, solvent flow rate, buffer concentration, orifice voltage, and interface distance. The collision energy was also adjusted for generation of daughter ions. Regarding HPLC conditions, a gradient elution of the brighteners with a mixture of acetonitrile and water containing ammonium acetate gave an acceptable chromatographic separation with the specified C_8 HPLC column.

The LC/MS conditions for stilbene and biphenyl stilbene derivatives were then studied in the negative-ion detection mode. Almost all brighteners found in commercial detergents belong to these two classes. Figure 4 shows HPLC/MS/UV/fluorescence chromatograms of a standard solution containing six stilbene derivatives and one biphenyl stilbene derivative. Under the conditions specified, 70-ppm (wt/vol) solutions of stilbene and biphenyl stilbene derivatives can be identified based on their mass spectra. Overlapping peaks (c, d, and e) are easily differentiated from each other by MS. Figure 5 shows the respective extracted ion chromatograms for the three overlapping peaks, providing individual information on each of the three brightener species.

Figure 6 shows the LC/MS/UV/fluorescence chromatograms for ten brighteners from the five classes of brighteners. The positive-ion mode was used on the MS for detection with this separation. Pyrazoline, oxazole, and coumarin derivatives

TABLE 1
Relative Ion Intensity Among Matrices

			Relative ion intensity		
Compound	Type	Ion mode	Acetonitrile/water (95:5)	Acetonitrile/water (50:50)	Acetonitrile/water containing 5mM ammonium acetate (50:50)
II	Stilbene	Negative	100	15	50
VII	Biphenyl stilbene	Negative	100	5	15
VIII	Pyrazoline	Positive	100	80	70
IX	Oxazole	Positive	100	70	1300
X	Coumarin	Positive	100	3	130

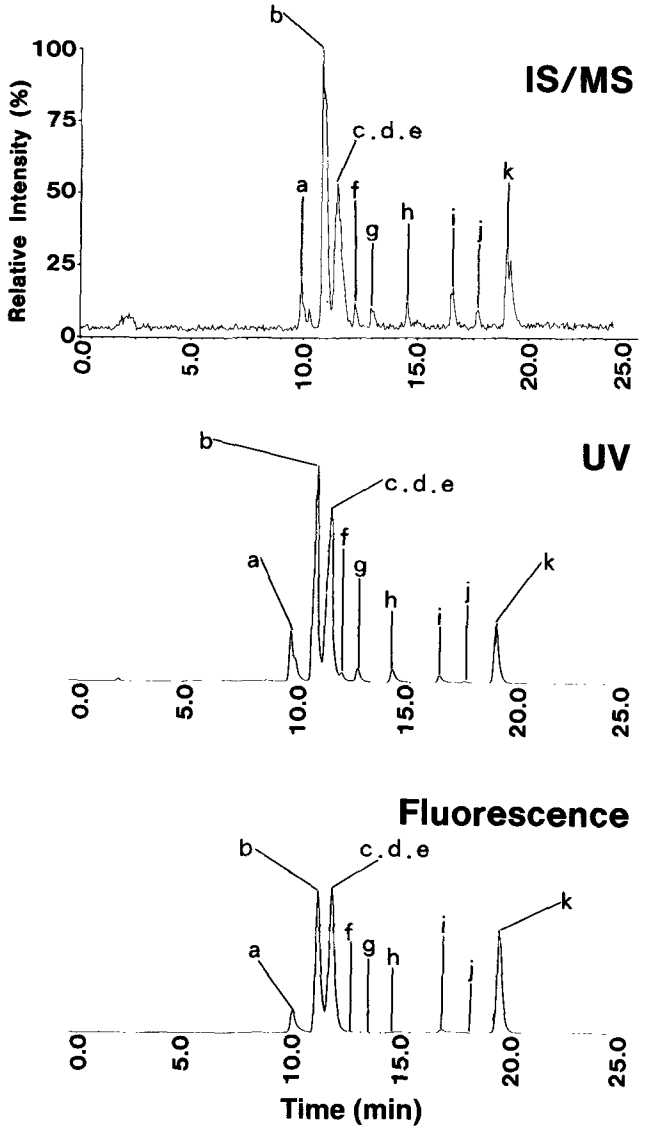

FIG. 4. High-performance liquid chromatography/mass spectrometry/ultraviolet/fluorescence chromatograms for stilbene and biphenyl stilbene derivatives in the negative-ion mode: a: **II** *trans,* b: **VII**, c: **I** *trans,* d: **III** *trans,* e: **IV** *trans,* f: **III** *cis,* g: **I** *cis,* h: **IV** *cis,* i: **V** *trans,* j: **V** *cis,* k: **VI**.

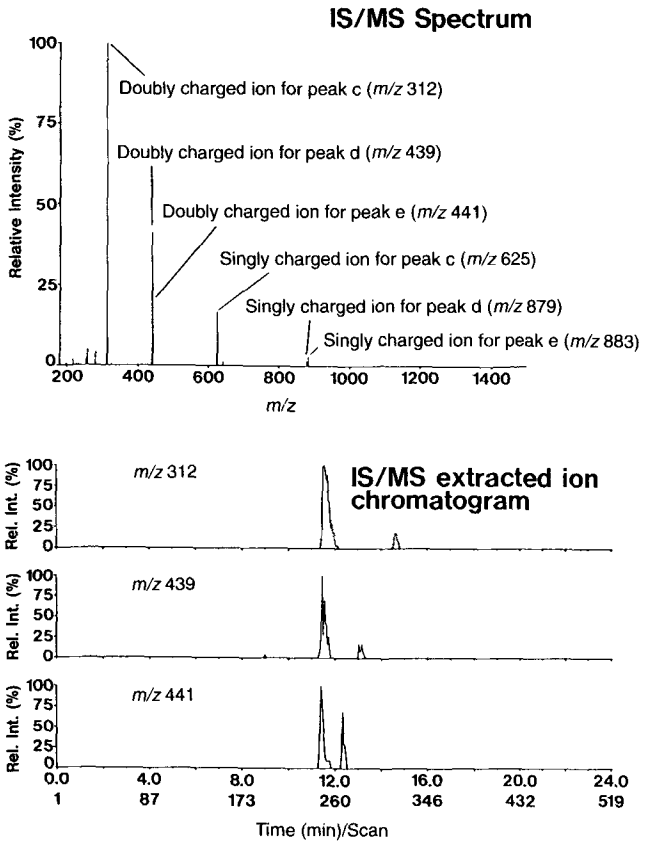

FIG. 5. Ion-spray/mass spectrometry (IS/MS) spectrum and extracted ion chromatograms for overlapping peaks (c, d, and e) in the high-performance liquid chromatography/MS chromatogram shown in Figure 4; Rel. Int., relative intensity.

were separated under the HPLC conditions specified and monitored with the UV, fluorescence, and MS detectors.

Identification. Figure 7 shows chromatograms of a commercial granular detergent A containing unidentified brighteners. The UV and fluorescence detectors positively located the brightener peaks in the LC/MS chromatogram. Figure 8 shows the IS/MS spectra for the corresponding peaks in the LC/MS chromatogram. Based on the results shown in Figure 8A (single-charged ion $m/z = 517$ and double-charged ion $m/z = 258$), one of the brighteners used in detergent A is brightener VII shown in Figure 1. Based on the spectra shown in Figure 8B, the other brightener used in this detergent was identified as a stilbene derivative. For more information, IS/MS/MS was conducted on this brightener. The mechanism of the daughter-ion generation is shown in Figure 9. Thus, the unknown brightener in detergent A was determined to have the molecular structure shown in Figure 9.

Almost all brighteners in detergents are stilbene and biphenyl stilbene derivatives (10). They elute before linear alkyl benzene sulfonate (LAS) and soap in most cases and can be identified by IS/MS and IS/MS/MS like the example reported above. When peaks of brighteners overlap with LAS and soap in the mass chromatogram, subtracting mass peaks of LAS or soap provides the mass spectra for targeted brighteners. First, the retention times for brighteners are specified by UV and fluorescence detectors. Then, mass spectra of corresponding retention times are taken. Because mass spectra for LAS and soap are known and show simple molecular ions, their mass spectra are easily recognized and can be subtracted from the original mass spectra, providing pure brighteners' spectra.

Detectors. The *trans-cis* isomerization in stilbene derivatives was studied with the HPLC/MS/UV/fluorescence system. The UV detector and mass spectrometer detect both *trans* and *cis* isomers. However, the fluorescence detector is insensitive to the *cis* isomer because the fluorescence from this isomer is weak. Figure 10A shows the UV and fluorescence chromatograms for brighteners I and V. These bright-

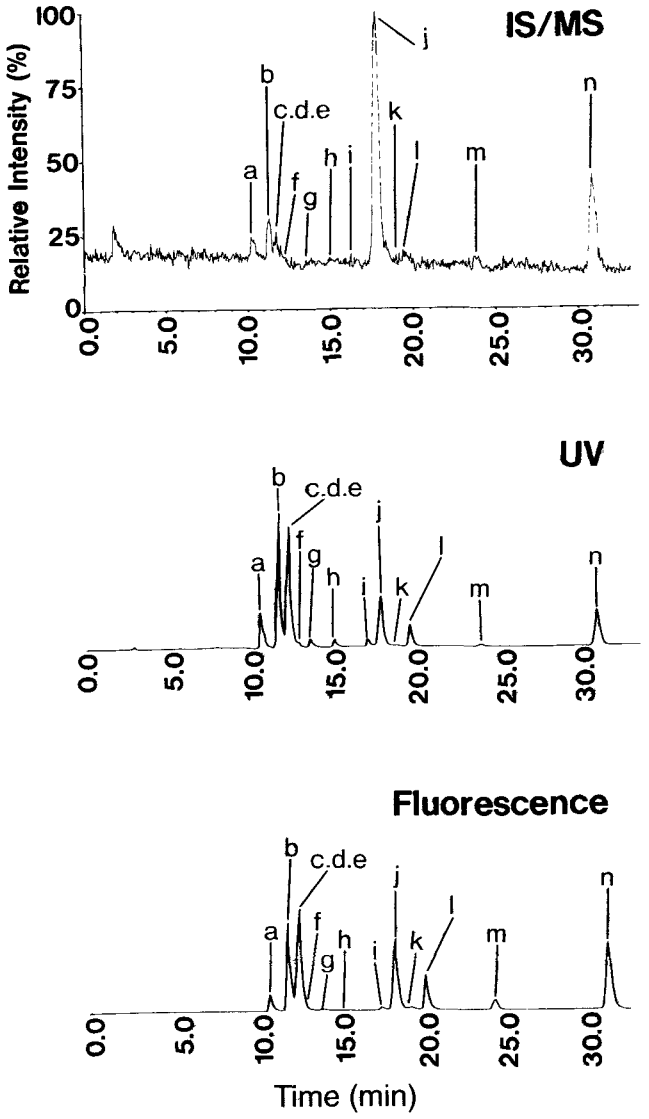

FIG. 6. High-performance liquid chromatography/MS/ultraviolet (UV)/fluorescence chromatogram for ten brighteners from five groups (stilbene, biphenyl stilbene, pyrazoline, oxazole, and coumarin derivatives) in the positive-ion mode; a: **II** *trans*, b: **VII**, c: **I** *trans*, d: **III** *trans*, e: **IV** *trans*, f: **III** *cis*, g: **I** *cis*, h: **IV** *cis*, i: **V** *trans*, j: **V** *cis*, k: **VI**, l: **X**, m: **VIII**, n: **IX**. See Figure 5 for abbreviations.

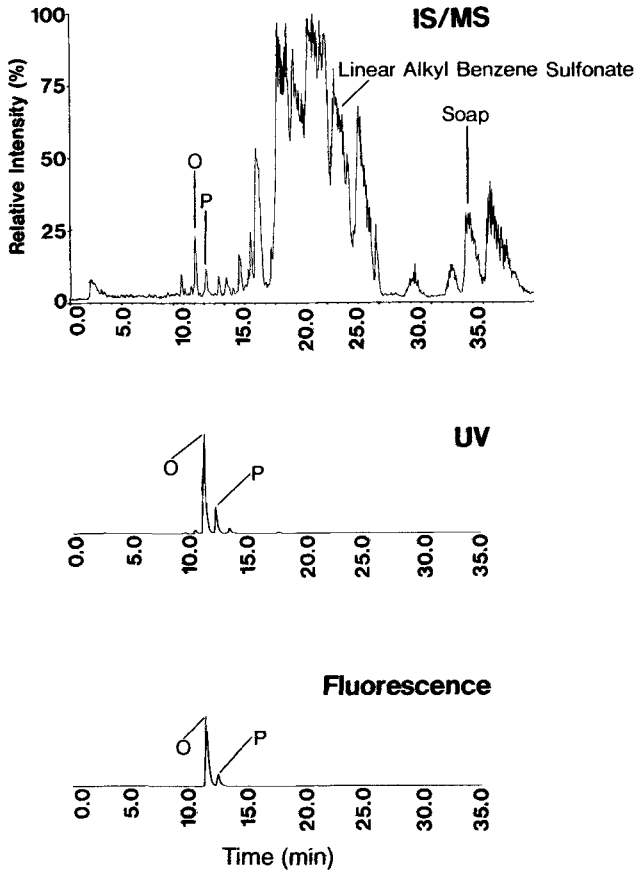

FIG. 7. High-performance liquid chromatography/MS/UV/fluorescence chromatograms for detergent A in the negative-ion mode. See Figures 5 and 6 for other abbreviations.

eners contain both *trans* and *cis* isomers. After one-week storage in the solvent of acetonitrile and water containing 0.1M ammonium acetate (50:50), the same sample was reanalyzed (Fig. 10B). The chromatograms show that the *cis* peaks increased in concentration. This was caused by the isomerization of the *trans* form to the *cis* form in stilbene derivatives due to the exposure to light (2,10,11,25). In this way, *trans* and *cis* isomers of stilbene derivatives can be easily differentiated from each other without standards.

Figure 11 shows the HPLC chromatogram and spectra for three overlapping peaks obtained by three-dimensional HPLC with a diode-array detector. Information on peak purity or resolution of overlapping peaks can be obtained by the diode-array detector. Routine quantitation of brighteners should be done with a diode-array detector, so that peak purity can be confirmed and overlapping peaks can be resolved.

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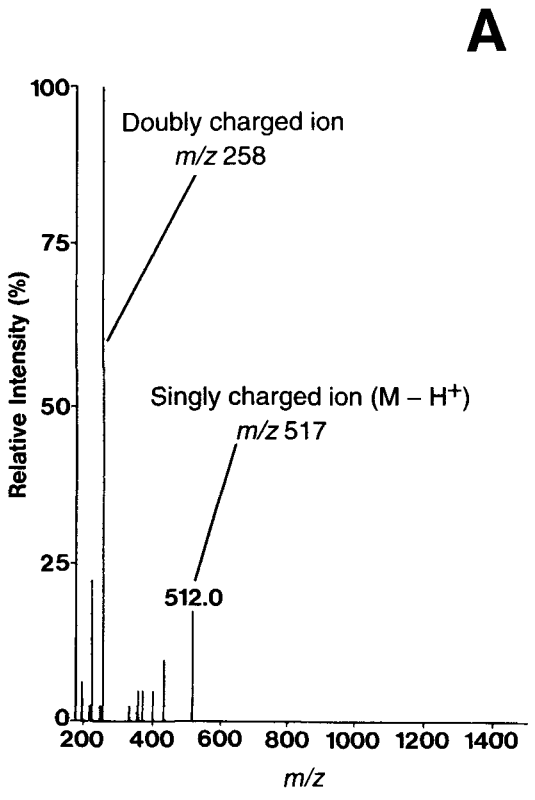

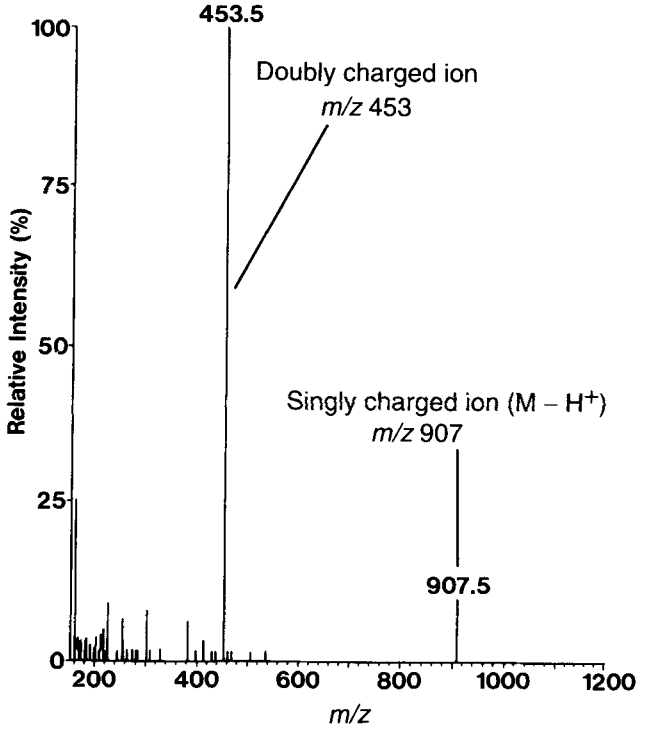

FIG. 8. Ion-spray/mass spectrometry spectrum for Peaks O and P shown in Figure 7. Detection was accomplished in the negative-ion mode. A = Peak O; B = Peak P.

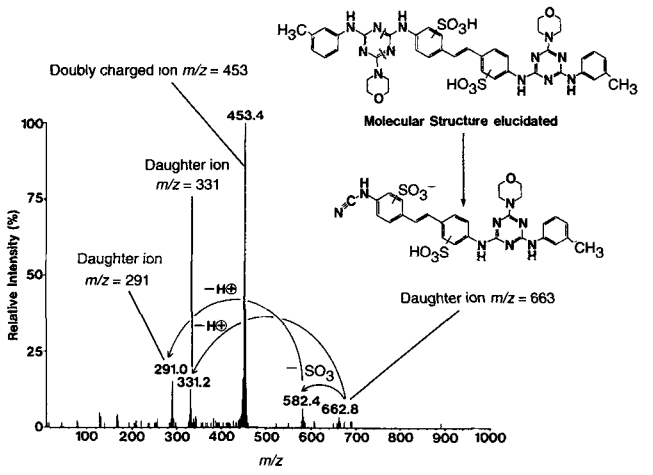

FIG. 9. Ion-spray/mass spectrometry (MS)/MS spectrum for *m/z* 453 from Peak P shown in Figure 7.

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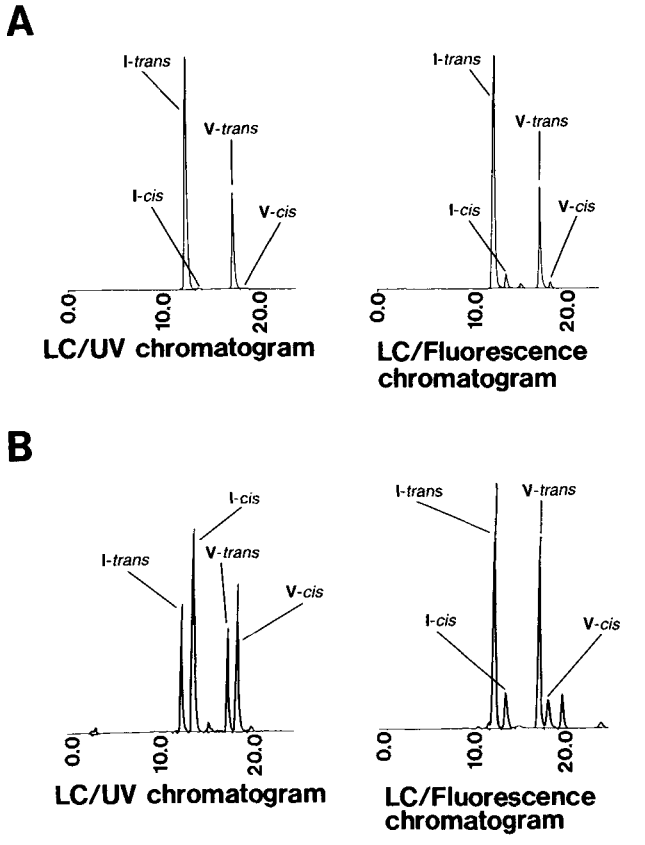

FIG. 10. Liquid chromatography (LC)/UV/fluorescence chromatograms for brighteners **I** and **V**. Sample solution was analyzed immediately after sample preparation (A) and one week later (B). See Figure 6 for other abbreviation.

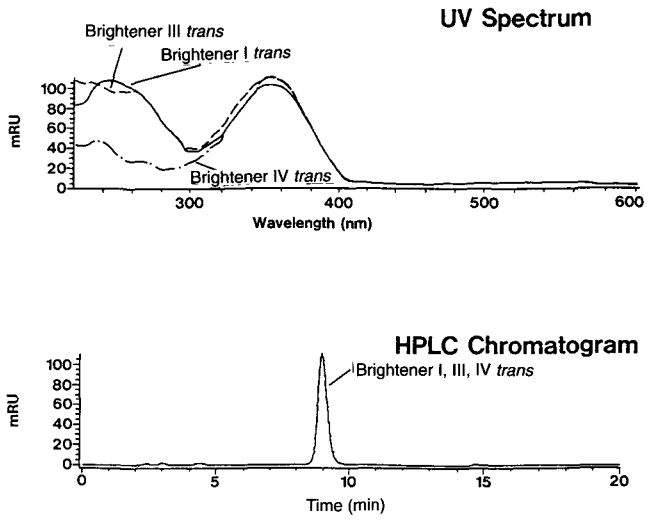

FIG. 11. Three-dimensional high-performance liquid chromatography (HPLC) spectrum and chromatogram for overlapping peaks (c, d, and e). See Figure 6 for other abbreviation.

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