

## Note

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### High-performance thin-layer chromatography of fluorescent whitening agents and their identification in detergents

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The fluorescent whitening agents (FWAs) are widely employed in the production of detergents, textiles, papers and plastics<sup>1</sup>. Such compounds are present at 0.1–0.6% levels in detergents and are of interest because of their possible rôle as dermal sensitizers. The identification of individual FWAs has become more and more difficult due to their increasing presence and their different structures.

Most relevant analytical studies have been performed by thin-layer<sup>2–7</sup> and column<sup>8,9</sup> chromatography on silica gel as the stationary phase. Attempts to use silanized silica gel columns generally gave satisfactory results<sup>10–12</sup>.

As thin-layer chromatography (TLC) is particularly suitable for the analysis of FWAs, we made a study on twenty whitening agents representing the major structural classes using high-performance TLC on ready-for-use plates of silanized silica gel with aqueous and non-aqueous solutions as eluents.

#### EXPERIMENTAL

The examined FWAs along to the following classes: (a) stilbene derivatives; (b) 1,3-diphenyl-3-pyrazolines; (c) coumarin and carbostyryl compounds; (d) benzoxazolyl substitution products of thiophene; (e) naphthalimides. The structures of the twenty compounds are shown in Fig. 1. Standard solutions were obtained by dissolving FWAs in water-methanol (1:1, v/v) and were stored in dark coloured bottles. Exceptions are compounds 13, 14, 17–20 which were dissolved in tetrahydrofuran. The concentration of the solutions was 0.01 mg/ml, the exception for compounds 2 (0.05 mg/ml) and 3 (0.02 mg/ml).

The amount of FWAs deposited on the layer is reported in Table I. Ready-for-use plates of Sil C<sub>18</sub>-50 UV<sub>254</sub> (Macherey-Nagel) and RP-2, RP-8 and RP-18 F<sub>254</sub> (Merck) were used. The migration distance was 6 cm, unless otherwise stated. All the measurements were carried out at 25°C using a Desaga thermostat-chamber protected from light. The spots were visualized under UV light (366 nm).

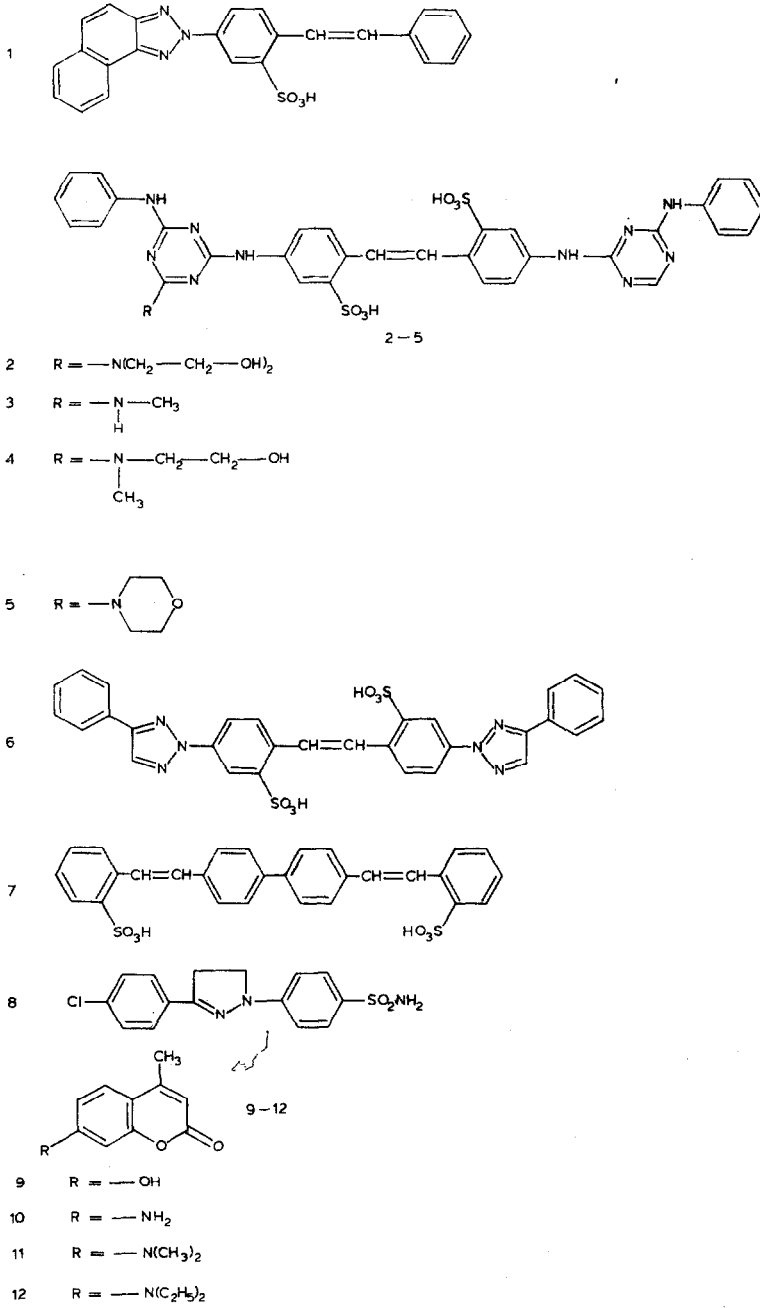


Fig. 1.

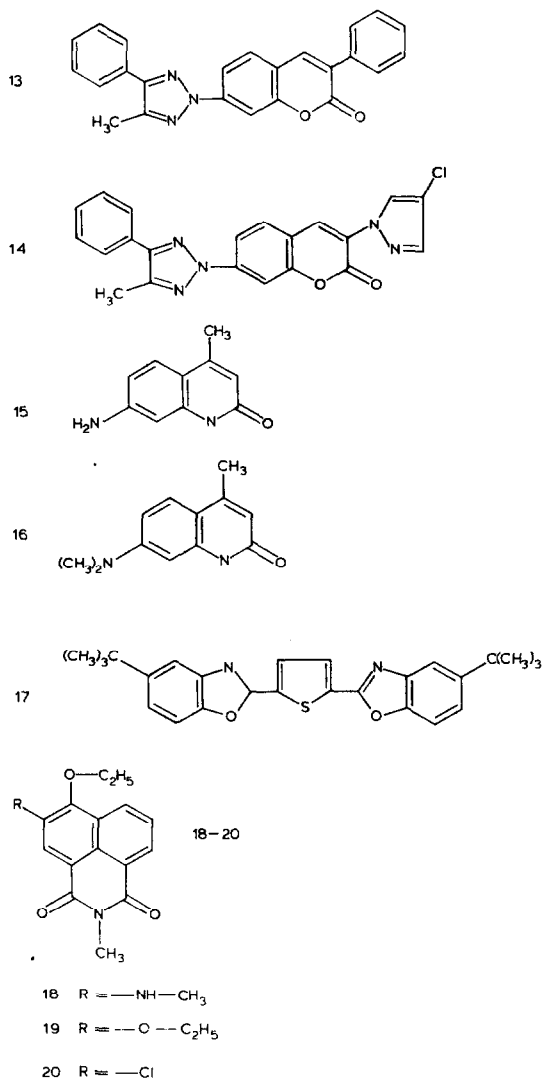


Fig. 1. The structures of the FWAs: 1 = 4-naphthotriazolylstilbene-2-sulphonic acid; 2 = 4,4'-bis[4-anilino-6-bis(2-hydroxyethylamino)-1,3,5-triazin-2-ylamino]stilbene-2,2'-disulphonic acid; 3 = 4,4'-bis[4-anilino-6-methylamino-1,3,5-triazin-2-ylamino]stilbene-2,2'-disulphonic acid; 4 = 4,4'-bis[4-anilino-6-(2-hydroxyethyl)methylamino-1,3,5-triazin-2-ylamino]stilbene-2,2'-disulphonic acid; 5 = 4,4'-bis[4-anilino-6-morpholino-1,3,5-triazin-2-ylamino]stilbene-2,2'-disulphonic acid; 6 = 4,4'-bis[4-phenyl-1,2,3-triazol-2-yl]stilbene-2,2'-disulphonic acid; 7 = 4,4'-bis(2-sulphostyryl)biphenyl; 8 = 1-(4-amidosulphonylphenyl)-3-(4-chlorophenyl)-2-pyrazoline; 9 = 4-methyl-7-hydroxycoumarin; 10 = 4-methyl-7-aminocoumarin; 11 = 4-methyl-7-dimethylaminocoumarin; 12 = 4-methyl-7-diethylaminocoumarin; 13 = 3-phenyl-7-(4-methyl-5-phenyl-1,2,3-triazol-2-yl)coumarin; 14 = 3-(4-chloropyrazol-1-yl)-7-(4-methyl-5-phenyl-1,2,3-triazol-2-yl)coumarin; 15 = 7-amino-4-methylcarbostyryl; 16 = 7-dimethylamino-4-methylcarbostyryl; 17 = 2,5-bis(5-*tert.*-butylbenzoxazol-2-yl)thiophene; 18 = N-methyl-4-methylamino-5-ethoxynaphthalimide; 19 = N-methyl-4,5-diethoxynaphthalimide; 20 N-methyl-4-chloro-5-ethoxynaphthalimide.

TABLE I

 **$R_F$  VALUES OF FWAs ON THIN LAYERS OF SILANIZED SILICA GEL ELUTED WITH AQUEOUS AND NON-AQUEOUS ELUENTS**

Eluents: A, 1 M acetic acid + 3% KCl in water-methanol (40%); B, 1 M acetic acid + 0.5 M HCl in water-methanol (40%); C, 1 M acetic acid + 3% KCl in water-methanol (60%); D, 1 M acetic acid + 0.5 M HCl + 3% KCl in water-methanol (60%); E, *n*-hexane-ethyl acetate-acetic acid (95:4:1); F, *n*-hexane-ethyl acetate-acetic acid (90:9:1).

FWA*	Sil C <sub>18</sub> -50		RP-2	RP-8	RP-18	RP-18	RP-8		RP-18	Sensitivity (ng)
	A	B	C	C	C	D	E	F	F	
1	0.00	0.00	0.25	0.07	0.04	0.04	0.00	0.00	0.00	5
2**	0.06	0.20	0.65	0.60	0.72	0.73	0.00	0.00	0.00	25
3	0.03	0.09	0.51	0.47	0.54	0.57	0.00	0.00	0.00	10
4**	0.04	0.10	0.52	0.48	0.57	0.61	0.00	0.00	0.00	5
5**	0.02	0.05	0.42	0.34	0.27	0.48	0.00	0.00	0.00	5
6	0.00	0.01	0.42	0.25	0.19	0.20	0.00	0.00	0.00	5
7	0.01	0.02	0.50	0.36	0.24	0.25	0.00	0.00	0.00	5
8	0.04	0.05	0.43	0.30	0.26	0.26	0.04	0.17	0.07	5
9	0.32	0.36	0.66	0.65	0.67	0.65	0.15	0.40	0.13	5
10	0.33	0.64	0.71	0.70	0.71	0.88	0.04	0.16	0.05	5
11	0.11	0.48	0.47	0.32	0.29	0.59	0.33	0.57	0.18	5
12	0.06	0.62	0.38	0.20	0.20	0.79	0.49	0.77	0.30	5
13	0.00	0.00	0.09	0.03	0.00	0.00	0.98	0.98	0.69	3
14	0.00	0.00	0.09	0.02	0.00	0.00	0.98	0.98	0.69	3
15	0.39	0.66	0.76	0.74	0.77	0.91	0.01	0.07	0.02	5
16	0.15	0.63	0.51	0.36	0.31	0.79	0.15	0.33	0.07	5
17	0.00	0.00	0.00	0.00	0.00	0.00	0.98	0.98	0.87	3
18	0.05	0.06	0.39	0.18	0.18	0.18	0.08	0.24	0.03	2
19	0.04	0.04	0.32	0.10	0.09	0.08	0.39	0.68	0.21	5
20	0.02	0.03	0.28	0.09	0.06	0.04	0.72	0.90	0.40	5

\* The numbers refer to the compounds in Fig. 1.

\*\* Gives a fluorescent secondary spot ( $R_F = 0.00$ ), whose intensity increases with increasing FWA concentration.

## RESULTS AND DISCUSSION

In order to determine the influence of the degree of silanization of the stationary phase and of the hydrophobic chain length on the chromatographic behaviour of FWAs, ready-for-use plates of Sil C<sub>18</sub>-50, RP-2, RP-8 and RP-18 were used.

*Sil C<sub>18</sub>-50 plates*

The plates have a 50% silanization degree and allow the use of eluents with a higher percentage of water with respect to those manufactured by Merck. For this reason the chromatographic data on Sil C<sub>18</sub>-50 layers were obtained by eluting with solutions A and B of Table I, that is with 1 M acetic acid + 3% potassium chloride in water-methanol (40%) and 1 M acetic acid + 0.5 M hydrochloric acid in water-methanol (40%). The presence of potassium chloride in eluent A results in more compact spots and, therefore, a better resolution compared with the elution without the salt. The  $R_F$  values are similar in each case. Under these experimental conditions

only the carbostyryl and the less-condensed derivatives of coumarin migrate significantly. The retention of these compounds increases with increase in the hydrophobicity of the substituents R as shown by the chromatographic behaviour of 7-aminocoumarin and 7-aminocarbostyryl derivatives. Some stilbene FWAs move only slightly from origin but give rise both to a main spot and to a secondary spot which remains at the application point and shows a yellow fluorescence in the case of compounds 4 and 5 and a blue fluorescence, like that of the main spot, in the case of compound 2. These secondary spots cannot be ascribed to the *cis*-form of the stilbene derivatives because such forms are non-fluorescent; the corresponding spots can only be observed when the *cis*-form turns into the *trans*-form, as shown from measurements made with increasing amounts of compounds 2-5.

After exposure of the eluted plates to daylight for 1 h, a second or a third blue fluorescent spot appears whose  $R_F$  value is greater than that of the other spots.

The addition of hydrochloric acid to the eluent (solution B) allows a clear separation of carbostyryl compounds and of less-condensed coumarin derivatives from all the others. This can be attributed to the presence of protonated amino groups which decrease the affinity of these compounds towards the stationary phase. Under these experimental conditions the sulphonated compounds also show a higher mobility and are better separated. A further increase in the hydrochloric acid concentration does not affect the  $R_F$  values.

Elution with alkaline solutions, *i.e.*, 0.5 M ammonia + 0.5 M ammonium chloride in water-methanol (40%), gave similar results to those obtained with solution A. The one exception is compound 9, which exhibits the highest  $R_F$  value owing to the presence of a phenolic group. With the alkaline eluent it is possible to separate compounds 9-12, 15 and 16.

#### *RP-2, RP-8 and RP-18 plates*

The chromatographic data obtained on these three layers are compared in Table I (columns 3, 4 and 5). The eluent was 1 M acetic acid with 3% potassium chloride in water-methanol (60%). In comparison with the other two layers, RP-2 exhibits a lower retention power and allows the separation among the three naphthalimides and that of the two more-condensed coumarin derivatives from the highly hydrophobic compound 17. The highest selectivity for the sulphonated FWAs is possessed by RP-18. The RP-8 and RP-18 plates exhibit very similar behaviour and also show a better resolution power with respect to Sil C<sub>18</sub>-50 with the same eluent. For this reason we carried out our study with aqueous-organic mixtures as eluents on these two plates.

In column 6 of Table I, data obtained on RP-18 with 1 M acetic acid, 0.5 M hydrochloric acid and 3% potassium chloride in water-methanol (60%), are reported. The addition of hydrochloric acid to the eluent results in full resolution of the seven sulphonated FWAs and a notable increase in the mobility of those compounds that exhibit ready protonation of the amino groups in their molecules.

Fig. 2 shows the separations of the seven derivatives of stilbene and the three naphthalimides on plates of RP-18 eluted with solution D.

Attempts were made to determine the influence of the organic solvent on the chromatographic behaviour of FWAs. The use of ethanol in place of methanol produced interesting results as shown by the chromatogram in Fig. 3, obtained on RP-8

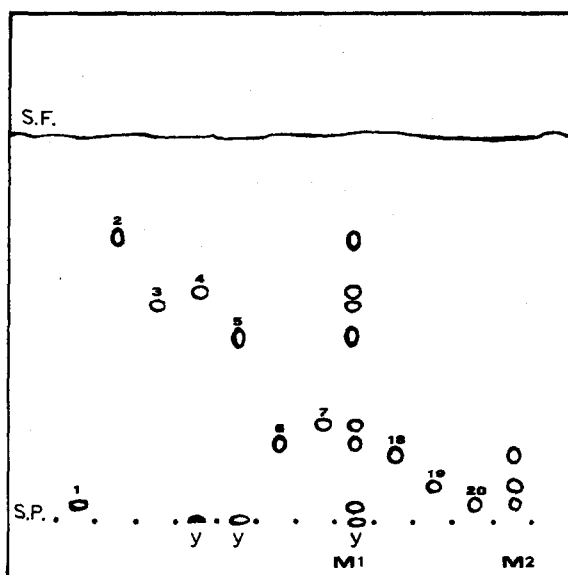


Fig. 2. Thin-layer chromatogram of FWAs on RP-18 plates. Eluent: 1 *M* acetic acid, 0.5 *M* HCl and 3% KCl in water-methanol (60%). Migration distance: 6.9 cm. y = Yellow fluorescence. The numbers refer to the compounds in Fig. 1. M<sub>1</sub> = Mixture of compounds 1-7; M<sub>2</sub> = mixture of 18-20. S.P. = Starting point; S.F. = solvent front.

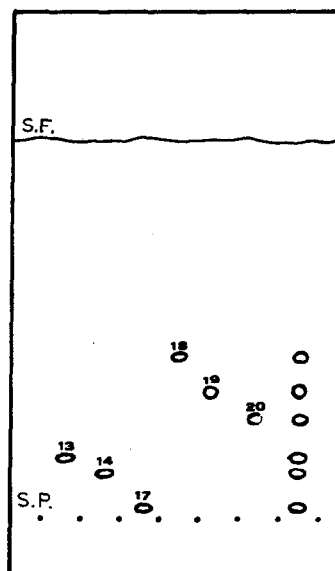


Fig. 3. Thin-layer chromatogram of the more hydrophobic FWAs on RP-8 plates. Eluent: 1 *M* acetic acid, 0.1 *M* HCl and 3% KCl in water-ethanol (60%). Migration distance: 6.8 cm.

plates eluted with 1 *M* acetic acid, 0.1 *M* hydrochloric acid and 3% potassium chloride in water-ethanol (60%). The clear separation among compounds 13, 14, 17-20 and particularly, that between the first two, not obtainable with methanol, should be noted. With such an eluent the other FWAs migrate much further than the compounds in Fig. 3 and do not interfere with the separation. The only exception is compound 1, whose  $R_F$  value is a little higher than that of compound 19.

#### *Non-aqueous eluents*

The use of non-aqueous eluents allows silanized silica gel to behave as a polar stationary phase owing to the presence of free hydroxyl groups<sup>13,14</sup>. Table I shows the data obtained on RP-8 and RP-18 plates eluted with mixtures of *n*-hexane, ethyl acetate and glacial acetic acid in different ratios (solutions E and F). Under these experimental conditions the chromatographic behaviour of FWAs is completely different from that found on the same plates with aqueous solutions. The elution time is only 15 min. The presence of acetic acid in the eluent, even at 1%, accounts for the compactness of the spots. An increase in ethyl acetate percentage results in an increase in the eluent polarity and in the  $R_F$  values of non-sulphonated FWAs.

The sulphonated compounds always remain at the application point because of their high polarity and can therefore be separated from all the others. The non-sulphonated FWAs, on the contrary, migrate according to their hydrophobic characteristics. The affinity sequence of the carbostyryl and coumarin derivatives and of the naphthalimides is opposite to that found with aqueous-organic solvents.

By eluting with solution F, the higher retention shown on RP-18 plates in comparison with RP-8 can be ascribed to the longer hydrophobic chain of the former.

With non-aqueous eluents, several interesting separations can be effected. In particular, it is possible to separate ten compounds with solution F on RP-8 plates (see Fig. 4). The same figure also shows the separation of the thirteen non-sulphonated FWAs by using a two-dimensional technique. After the first elution with solution F, the plate was dried at room temperature for 1 h in order to allow complete evaporation of the solvents.

#### *Analysis of detergents*

For the extraction of whitening agents, the commercial products (250 mg) were stirred with 10 ml of water-methanol (1:1 v/v). A 0.5- $\mu$ l volume of this solution was deposited on the plate.

On RP-8 layers the use of the solution F allows the determination of non-sulphonated whitening agents in the detergents, since all the sulphonated compounds remain at the application point and do not interfere with the identification of the former group. Only Lava Sbianca detergent shows a main spot ( $R_F = 0.57$ ) due to whitening agent 11 (4-methyl-7-dimethylaminocoumarin). Tests performed on detergents extracted with dimethylformamide did not reveal the presence of the more hydrophobic compounds, such as 13, 14 and 17, which dissolve in such a solvent.

Fig. 5 shows the chromatographic behaviour of the whitening agents contained in the detergents examined on layers of RP-18 eluted with 1 *M* acetic acid, 0.5 *M* hydrochloric acid and 3% potassium chloride in water-methanol (60%). From this chromatogram and from further tests performed under different experimental conditions on RP-8 and RP-18 plates in the presence of standard FWAs, the whitening

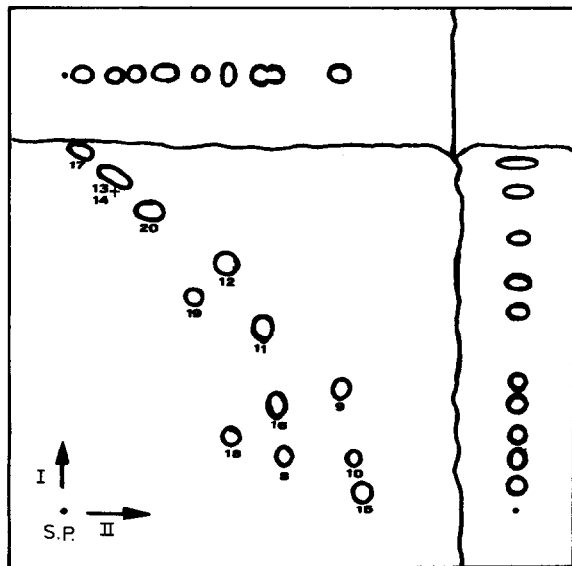


Fig. 4. Two-dimensional chromatogram of non-sulphonated FWAs on RP-8 plates. Eluents: first direction, *n*-hexane-ethyl acetate-acetic acid (90:9:1) (solution F); second direction, 1 *M* acetic acid and 3% potassium chloride in water-ethanol (60%).

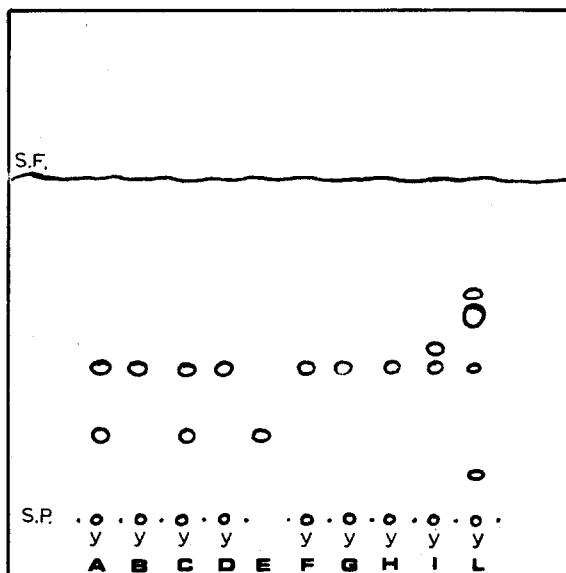


Fig. 5. Thin-layer chromatogram on RP-18 plates of commercial detergents extracted with water-methanol. Eluent: 1 M acetic acid, 0.5 M HCl and 3% KCl in water-methanol (60%). A = Sole bucato; B = Ariel; C = Ava; D = Esselunda; E = Omo; F = Scala; G = Lanza Lavatrici; H = Bingo; I = Ola'; L = Lava Sbianca.

agents in the commercial products are generally compound 5 or 5 together with 7. The exceptions are the detergents OLA' and LAVA SBIANCA which contain, respectively, compound 5 with a fluorescent unknown compound and numbers 5 and 11 with two other unknown fluorescent compounds.

#### ACKNOWLEDGEMENTS

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#### REFERENCES

- 1 R. Anliker and G. Muller, in F. Coulston (Editor), *Fluorescent Whitening Agents, Suppl. Vol. IV, Environmental Quality and Safety*, Thieme, Stuttgart, 1975, pp. 47-65.
- 2 J. Latinák, *J. Chromatogr.*, 14 (1964) 482.
- 3 H. Theidal and G. Schmitz, *J. Chromatogr.*, 27 (1967) 413.
- 4 K. Figge, *Fette, Seifen, Anstrichm.*, 70 (1968) 680.
- 5 J. Schulze, T. Polarco and P. S. Stensby, *Soap, Cosmet., Chem. Spec.*, 50 (1974) 46.
- 6 J. B. F. Lloyd, *J. Forensic Sci. Soc.*, 17 (1977) 145.
- 7 A. H. Lawrence and D. Ducharme, *J. Chromatogr.*, 194 (1980) 434.
- 8 D. Kirkpatrick, *J. Chromatogr.*, 121 (1976) 153.
- 9 D. Kirkpatrick, *J. Chromatogr.*, 139 (1977) 168.
- 10 B. P. McPherson and N. Omelczenko, *J. Amer. Oil Chem. Soc.*, 57 (1980) 388.
- 11 N. Atsuo, M. Morita and Y. Makoto, *Bunseki Kagaku (Jap. Anal.)*, 29 (1980) 69.
- 12 G. Micali, P. Curro' and G. Calabrò', *Proceedings of the First International Symposium on "Technological, Environmental and Economic Trends on Detergency"*, Rome, October 22-24, 1980, Vol. II, Institute of Commodity Science, Rome, 1980, p. 234.
- 13 L. Lepri, P. G. Desideri and D. Heimler, *J. Chromatogr.*, 260 (1983) 383.
- 14 L. Lepri, D. Heimler and P. G. Desideri, *J. Chromatogr.*, 288 (1984) 461.