Journal of Chromatography, 255 (1983) 101–114
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,301

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF QUINOIDAL IMMINIUM COMPOUNDS DERIVED FROM TRIPHENYLMETHANES

S. L. ABIDI
U.S. Fish & Wildlife Service, Department of Interior, P.O. Box 65, La Crosse, WI 54601 (U.S.A.)

SUMMARY

A series of eleven *p*-aminotriphenylmethane dyes have been studied by high-performance liquid chromatography (HPLC). The combined use of HPLC and spectrophotometry permits specific detection of these compounds in the visible range around 600 nm. As the high affinity of the imminium cations for the active sites of the hydrocarbonaceous stationary phase has presented difficulties for reversed-phase HPLC with pure solvents, organic electrolytes were added to the mobile phase to facilitate the elution of the components with improved selectivity, sensitivity (minimum detection limit, 0.1 μg/ml), and peak symmetry. The effects of chromatographic variables on the component retentivity were investigated. Retention times of the dye analytes decreased with increasing concentration of the added ionic reagent and with decreasing number of the hydrophobic alkyl substituents on the nitrogen atom. The influence of pH on the retention parameters appears to parallel that observed previously for cationic quaternary ammonium compounds. Among the acidic reagents employed, naphthalenesulfonic acid yielded the most satisfactory results. The use of binary electrolyte systems invariably improved the chromatographic behavior of the imminium solutes analyzed. Results obtained with two different octadecylsilica columns have been compared.

INTRODUCTION

Quinoidal imminium compounds derived from triphenylmethanes are widely used as coloring agents in the fabric and leather industries. Many of the familiar biological staining materials are members of this family of dye substances. In addition, the significant biological activity of the majority of these compounds (antibacterial and antifungal[1,2]) and in particular of the N,N,N′,N′-tetramethylaminotriphenylmethane dye, malachite green (MG) (carcinogenic, mutagenic, and teratogenic[3]) has provided impetus to the assessment of the impact of the usage of these chemicals on the environment. Our interest in analytical methodology pertaining to the dye residues in the aqueous environment arose from concern over the use of MG as an agricultural and fishery fungicide.

Until recently, there were only a few reports describing non-specific assays for triphenylmethane dyes by paper chromatography[4] and thin-layer chromatography[5]

0021-9673/83/0000–0000/$03.00

in conjunction with colorimetry. In an earlier paper[6], I presented a preliminary account of a portion of this work with emphasis on the application of high-performance liquid chromatography (HPLC) to the analysis of selected fungitoxic *p*-aminotriphenylmethanes along with the dihydro-leuco-bases (possible metabolites) in aqueous and organic tissue samples. This paper describes the systematic study of the HPLC behavior of a series of triphenylmethane dyes (Figs. 1 and 2) each having a minimum of two *p*-amino groups with a positive charge localized on the nitrogen atom of the imminium moiety.

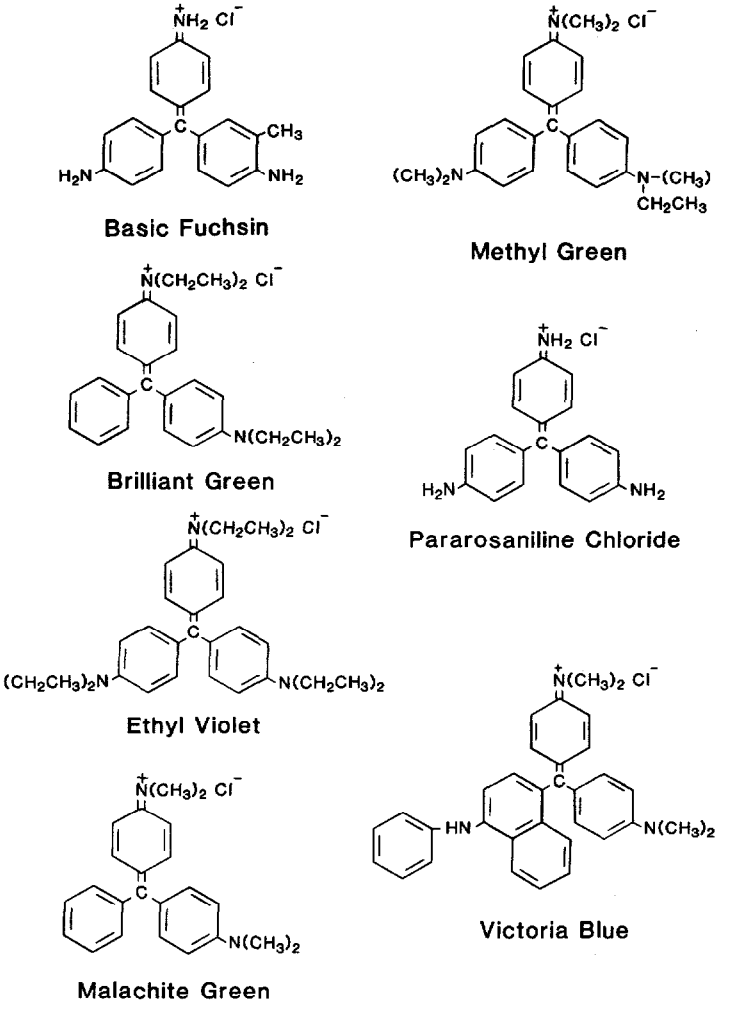

Fig. 1. Structures of the triphenylmethane dyes investigated.

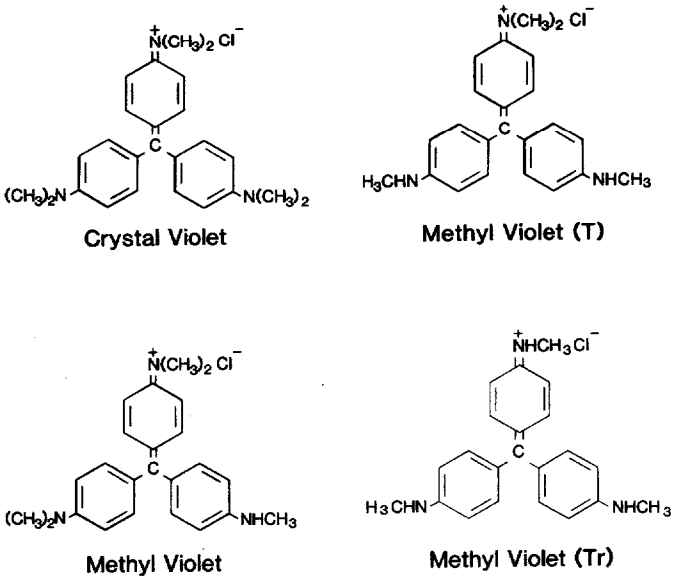

Fig. 2. Structures of methyl violet (MV) along with the N-methyl and N-desmethyl analogues of interest.

EXPERIMENTAL

Chemicals and reagents

Duplicate standard dye compounds were acquired from Aldrich (Milwaukee, WI, U.S.A.) and Eastman-Kodak (Rochester, NY, U.S.A.). Sodium methanesulfonate was prepared by treating the corresponding acid with a methanolic solution of sodium methoxide followed by recrystallization from methanol. Other sodium salts of alkane- and arenesulfonic acids used in this study were obtained from Eastman-Kodak and were used as supplied. Acetonitrile, methanol, and water for HPLC were of chromatography quality (Burdick & Jackson Labs., Muskegon, MI, U.S.A.). HPLC grade perchloric acid (70%), phosphoric acid (85%), and sulfuric acid were purchased from J. T. Baker (Phillipsburgh, NJ, U.S.A.). The perhalogenated acetic acids (trichloro- and trifluoro-) were ultrapure products of Eastman-Kodak. All other chemicals including buffer salts and solvents were analytical reagent materials.

High-performance liquid chromatography (HPLC)

A Varian Model 5000 liquid chromatograph equipped with a multiple wavelength UV–visible detector and a Varian Model 9176 strip chart recorder was used. Retention times were automatically determined by a Varian Model CDS III-L data system. Column effluents were monitored at 600 nm for multicomponent samples, whereas in calibration studies of samples involving a single analyte the detector was set at the absorption maximum, λ_{max} of the individual dyes. Sample components dissolved in acetonitrile at a level of 50 μg/ml were injected into an analytical column via an injector unit (composed of a Valco injector valve and a 10-μl loop) and a guard column (5 cm × 4 mm I.D.) packed with Vydac reversed-phase hydrocarbon (40 μm).

The performance of the two analytical columns containing octadecylsilica packings (5 μm) of different manufacturers' specifications was evaluated under identical HPLC conditions. One column (A) was custom-packed with Altex Ultrasphere (spherical forms with end-capping) and the other (column B) with Varian MicroPak-MCH (irregular forms without end-capping). Depending on the nature of the experiments, the composition of the mobile phase varied from 50% to 90% for acetonitrile and from 50% to 10% for water; the concentration of electrolyte reagents ranged from 0.005 to 0.15 *M*. The flow-rate of the isocratic elution was 2–3 ml/min, and the column was maintained at ambient temperature throughout the analyses.

RESULTS AND DISCUSSION

Because of their ready availability, the following N,N-dialkyl-, N-monoakyl-, and unsubstituted *p*-aminotriphenylmethanes of various lipophilicities were selected for this study: basic fuchsin (BF), brilliant green (BG), ethyl violet (EV), malachite green (MG), methyl green (MeG), pararosaniline chloride (PRC), victoria blue (VB) (Fig. 1), crystal violet (CV), methyl violet (MV), methyl violet-tetramethyl [MV(T)], and methyl violet-trimethyl [MV(Tr)] (Fig. 2). In the early stages of the investigation, the feasibility of employing either gas chromatography of the chemically reduced

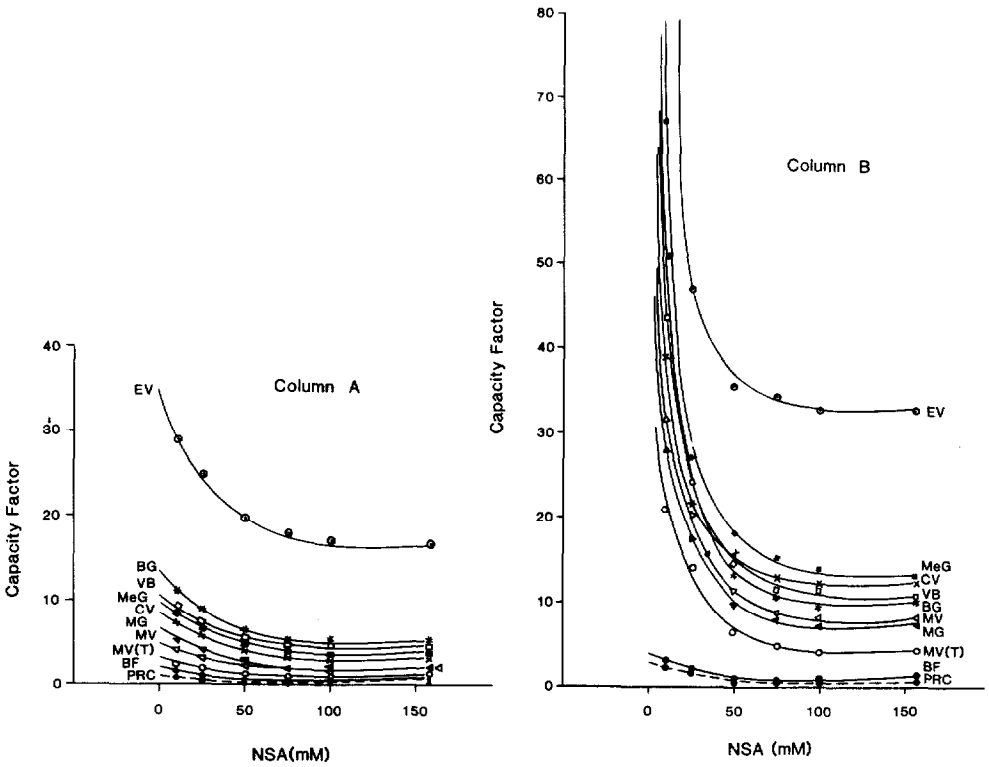

Fig. 3. Capacity factor, k', as a function of the NSA concentration in the presence of 0.01 *M* $HClO_4$. Mobile phase solvents: acetonitrile–water (70:30). Flow-rates under isocratic elution: column A, 2 ml/min; column B, 3 ml/min.

dihydro-dyes or HPLC with electrochemical detection was examined in view of the favorable low redox potentials of the triphenylmethane dyes, but there were problems associated with both methods.

From my experience with HPLC of high-molecular-weight quaternary ammonium salts[7], it was not unexpected that repeated attempts to elute the imminium salts through the reversed-phase column with mobile phases of acetonitrile (methanol)–water were thwarted in all cases owing to the strong adsorption of the imminium cations on the stationary phase. Fortunately, the addition of organic and inorganic electrolytes to the aforementioned mobile phase system greatly favored the elution of the dye components with improved selectivity, sensitivity, and peak symmetry. In order to obtain a better understanding of the chromatographic process, the influences of the counter ion concentration, the solvent composition, pH, column packings, the number of N-methyl groups, and the type of ionic reagents on the retention behavior of the quinoidal imminium dyes were investigated.

The effect of the concentration of naphthalenesulfonic acid (NSA) and perchloric acid on the capacity factor, k', is shown in Figs. 3 and 4, respectively. Retention values in the lower concentration range (0.005–0.075 M) appear to be more sensitive to concentration than those in the upper concentration region (0.075–0.15 M). The experiments involved isocratic elution with mobile phases consisting of a

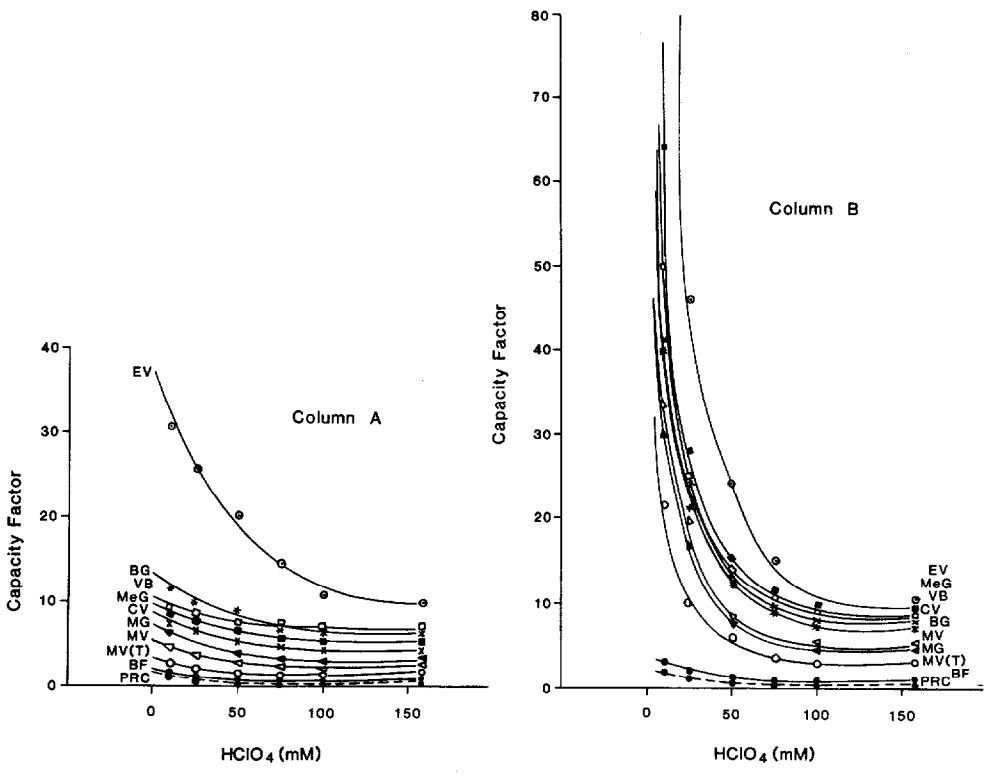

Fig. 4. Capacity factor, k', as a function of the $HClO_4$ concentration in the presence of 0.01 M NSA. Other HPLC conditions as in Fig. 3.

variable combination of NSA–$HClO_4$ in acetonitrile–water (70:30). Under these conditions, increase in the counter ion concentration generally lead to decreased k' values.

Fig. 5 illustrates the change in capacity factor with the change in composition of the mobile phase. Predictably, a decrease in retention of the dyes is observed as the proportion of water decreases resulting in decreasing surface tension of the mobile phase[8]. To demonstrate the dependence of the retention parameters upon pH, seven mobile phases comprising acetonitrile–water (70:30), 0.01 *M* $HClO_4$, and 0.1 *M* NSA at pH values in the range 2.5–7.0 were used (Fig. 6). The retention of the imminium solutes gradually increased with the rise in pH from 3 to 7. A rationale based on the ionic character of surface silanols[9] should probably be exploited to explain the observed retention behavior. The increased degree of ionization of the polar silanol groups with increasing pH from 3 to 7 would subject the analyte ions to attraction forces and thereby increase their retention. In other words, the cationic dye samples are more likely to adsorb on the octadecyl bonded silica surface at higher pH values.

From Figs. 3–6, a qualitative generalization with respect to the structure–retentivity relationship can be drawn. In reversed-phase HPLC of the quinoidal imminium compounds, generally the N-alkyl hydrocarbon side chain plays an important role in the retention process: the longer is the alkyl chain length or the higher the

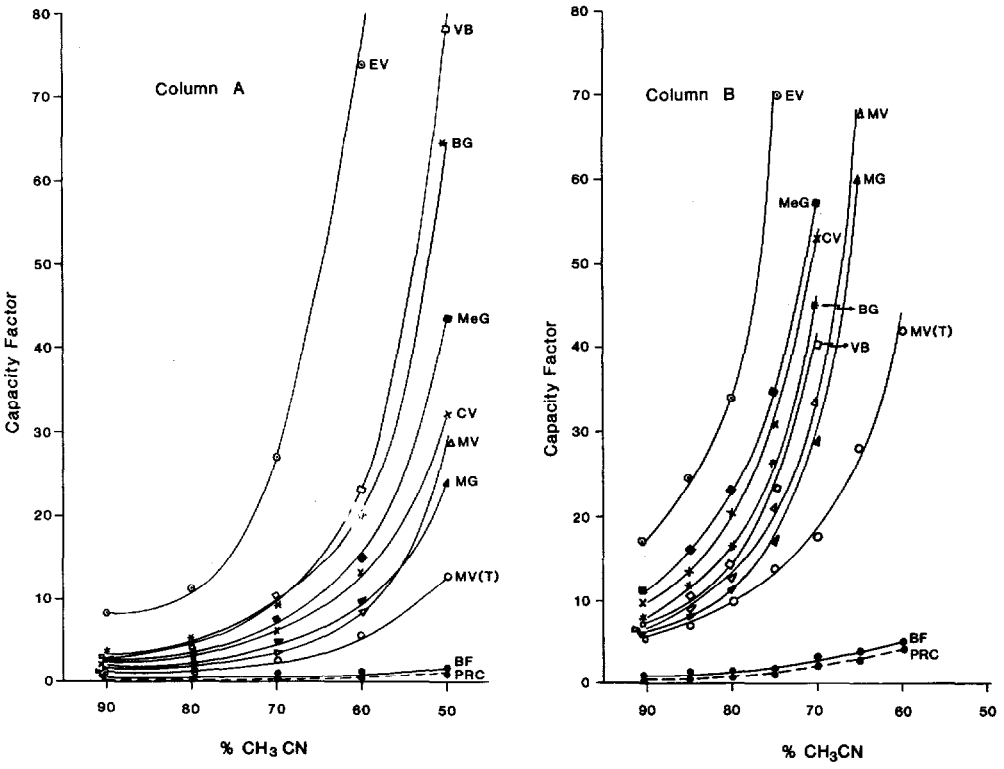

Fig. 5. Effect of the mobile phase solvent composition on k' in the binary (0.01 *M* $HClO_4$ + 0.01 *M* NSA) electrolyte system. Other HPLC conditions as in Fig. 3.

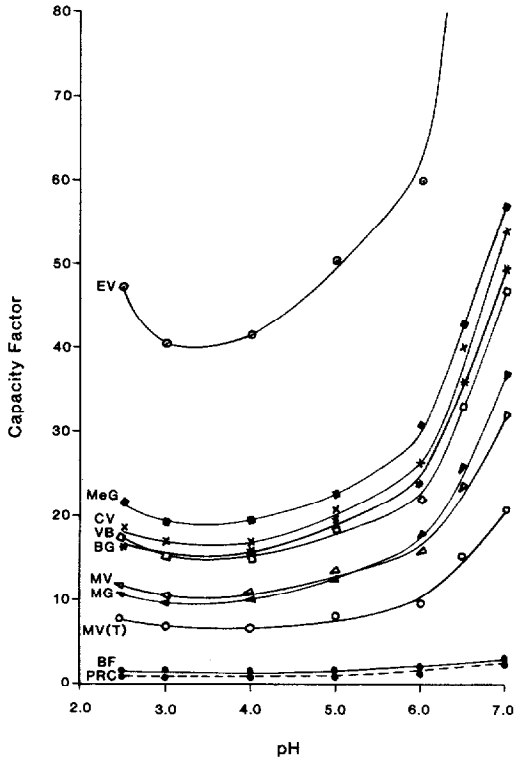

Fig. 6. pH dependence of the component retentivity, k', of the imminium dye mixture studied. Mobile phase: acetonitrile–water (70:30) containing 0.01 M $HClO_4$ and 0.01 M NSA with various amounts of K_2HPO_4. For all chromatographic runs, column B was used under isocratic elution at 3 ml/min.

number of N-alkyl groups, the longer is the retention time. The three phenyl groups in the triphenylmethane dyes seem to contribute little to the retention of the compounds as supported by the low retentivity of the N-unsubstituted substances (BF and PRC). Within the same type of dyes, the retention behavior can be well correlated with the hydrophobic character of the N-alkyl group. Thus, the three tris-N,N-dialkyl compounds are eluted in the order CV, MeG, and EV in accordance with the increasing hydrophobic N-alkyl chain length. Of the two bis-N,N-dialkyl compounds, the N,N-diethyl dye, BG, is more strongly retained than the N,N-dimethyl homologue, MG. With compounds of different types, there exist uncertainties in determining the retention sequence on the basis of merely the lipophilic structures especially when the difference in the total number of N-alkyl carbons is small. For example, studies of the retention curves (Figs. 3–6) for BG (8 N-alkyl carbons) and CV (6 N-alkyl carbons) revealed that reversal in the elution sequence (k' of CV is larger than that of BG) has occurred in some instances.

In connection with the above structure–retentivity study, the HPLC elution pattern of the analogous MV dyes is of interest. The commercial product labeled as methyl violet was analyzed by this HPLC method. It was found to contain at least four dye components whose structures are depicted in Fig. 2. Subjecting the samples of this material to HPLC analysis under various conditions afforded well resolved

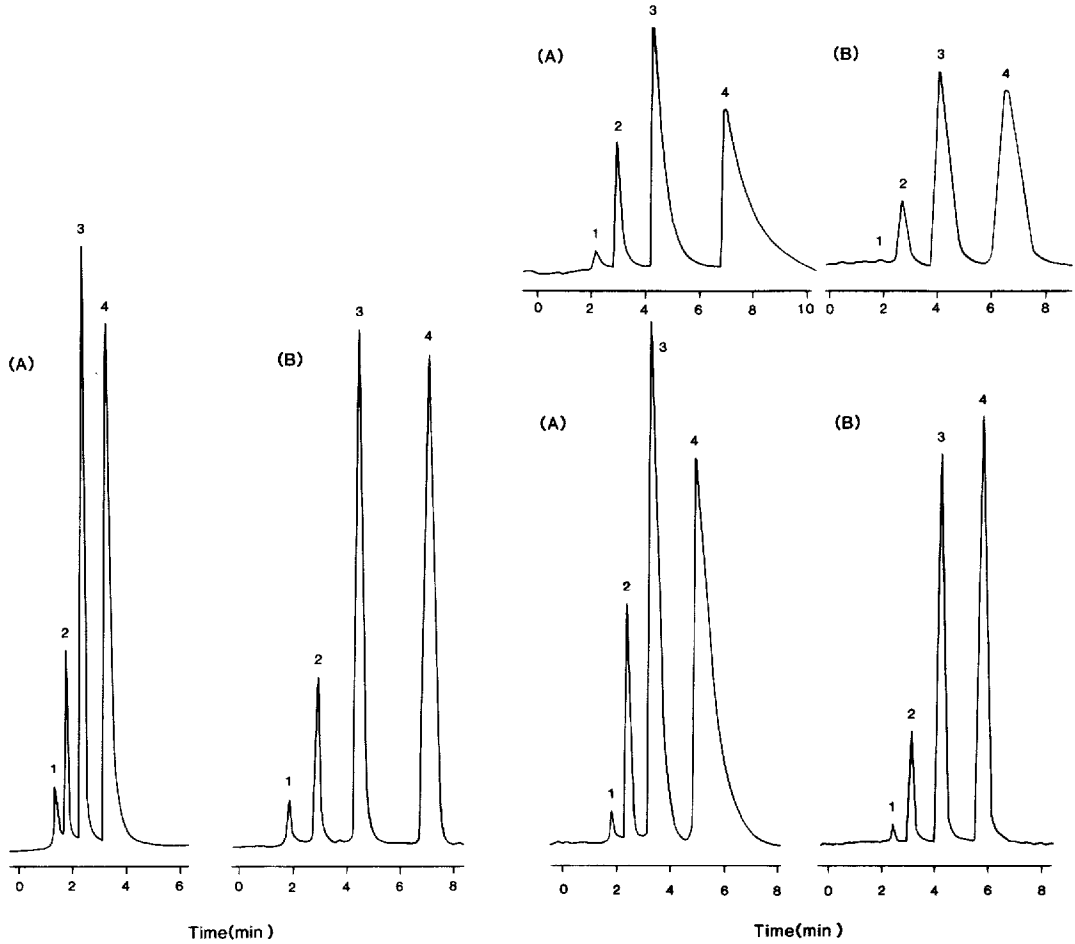

Fig. 7. HPLC separation of commercial MV samples. Components: 1 = MV(Tr); 2 = MV(T); 3 = MV; 4 = CV. HPLC conditions: mobile phase, acetonitrile–water (70:30) containing 0.01 *M* $HClO_4$ and 0.1 *M* NSA; flow-rates under isocratic elution, 2 ml/min with column A, 3 ml/min with column B.

Fig. 8. HPLC separation of commercial MV samples. Components as in Fig. 7. HPLC conditions: top chromatograms, acetonitrile–water (70:30) containing 0.01 *M* $HClO_4$ and 0.01 *M* BSA (sodium salt of benzenesulfonic acid); bottom chromatograms, acetonitrile–water (80:20) containing 0.01 *M* NSA and 0.01 *M* trifluoroacetic acid. Other chromatographic conditions for (A) and (B) as in Fig. 7.

chromatograms (Figs. 7 and 8). It is noteworthy that nearly half of the chromatograms (particularly those obtained with column A) exhibit some degree of peak tailing, while others display well-defined chromatographic peaks with excellent resolution. A logarithmic plot of the capacity factor *versus* the number of N-methyl groups in each of the MV analogues (Fig. 2) is shown in Fig. 9. The straight lines obtained can be expressed as

$$\ln k' = aN + b$$

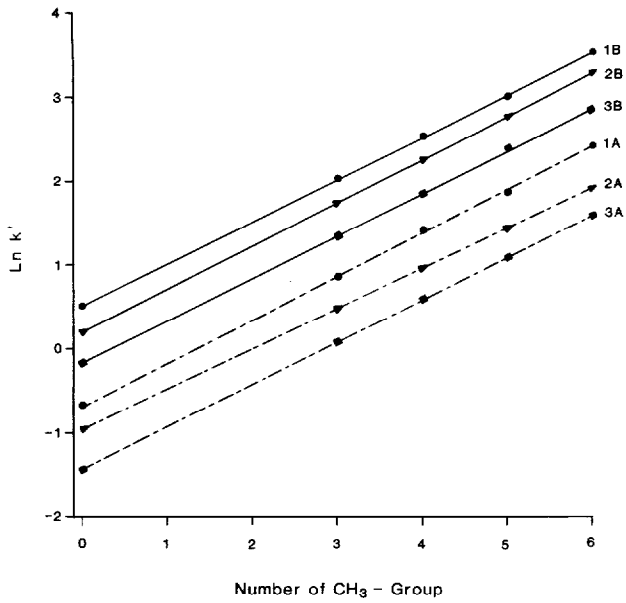

Fig. 9. Linear correlation of the logarithm of k' with the number of N-methyl groups in MV compounds (Fig. 2) including PRC. The HPLC conditions for lines 1A, 2A, 3A and 1B, 2B, 3B are as in Tables II and I respectively under the acetic acid group.

where k', N, a, and b represent respectively the capacity factor, the number of N-methyl groups in the MV component, the slope, and the intercept at $N = 0$. Table I summarizes the results of the correlation study, and also includes the data from analyses carried out with mobile phases other than those given in Fig. 9. The data show close agreement between the observed ln k' values for PRC and the intercept b values, suggesting the possibility of using linear correlations for the prediction of the retention values of structurally related unknown components.

In the course of a search for the most suitable counter ion reagent for this study, a variety of inorganic and organic acids and their binary mixtures were evaluated. Similarly to reversed-phase HPLC of quaternary ammonium salts[7], the use of mixed electrolytes improved the chromatographic characteristics of the imminium samples. To determine the effect of the type of acidic reagents on the component retentivity the capacity factor, k', was measured for all the compounds in the HPLC systems where numerous pairs of electrolyte reagents were employed. Table II shows the retention data obtained for the imminium dyes under HPLC conditions employing four different acid groups: alkanesulfonic acids, aromatic sulfonic acids, perhalogenated acetic acids (including acetic acid), and three inorganic acids ($HClO_4$, H_2SO_4, and H_3PO_4). Among these acids, NSA proved to be the most suitable for HPLC of the quinoidal imminium compounds, whereas acetic acid and phosphoric acid yielded less satisfactory results, which reflects the relatively weak acidity of both acids within their acid group. Fig. 10 shows some examples of chromatograms obtained for commercial MV samples using various binary electrolyte systems. The choice of MV compounds in this study was based solely on simplicity. The effect of the natures and structures of the added electrolyte reagents bears close relation to the

TABLE I

RELATION ($\ln k' = aN + b$) BETWEEN THE CAPACITY FACTOR, k', AND THE NUMBER, N, OF N–CH_3 GROUPS IN MV COMPOUNDS STUDIED UNDER VARIOUS MOBILE PHASE CONDITIONS

Column B was used under isocratic elution at a flow-rate of 3 ml/min; data were compared by varying one of the added reagents in the binary electrolyte systems. Abbreviations used: sodium salts of octanesulfonic acid (OSA), pentanesulfonic acid (PSA), methanesulfonic acid (MSA), benzenesulfonic acid (BSA), toluenesulfonic acid (TSA), and 2,4,6-trinitrobenzenesulfonic acid (TNBSA).

No.	*Mobile phase (acetonitrile–water, 70:30)*	*Slope, a*	*Intercept, b*	*ln k' (PRC)* (observed)*	*r***
Acetic acids					
1	0.01 *M* NSA + 0.1 *M* CH_3COOH	0.5158	0.5211	0.5149	0.997
2	0.01 *M* NSA + 0.1 *M* CCl_3COOH	0.5172	0.2050	0.1889	0.999
3	0.01 *M* NSA + 0.1 *M* CF_3COOH	0.5043	−0.1533	−0.1561	0.999
Alkanesulfonic acids					
1	0.1 *M* $HClO_4$ + 0.01 *M* OSA	0.4679	−0.5457	−0.5521	0.998
2	0.1 *M* $HClO_4$ + 0.01 *M* PSA	0.4842	−0.6900	−0.6885	0.999
3	0.1 *M* $HClO_4$ + 0.01 *M* MSA	0.4765	−0.8512	−0.8478	0.999
Aromatic sulfonic acids					
1	0.01 *M* $HClO_4$ + 0.01 *M* NSA	0.5024	0.4325	0.4404	0.999
2	0.01 *M* $HClO_4$ + 0.01 *M* BSA	0.5150	0.0121	0.0110	0.999
3	0.01 *M* $HClO_4$ + 0.01 *M* TSA	0.5057	−0.3016	−0.3130	0.998
4	0.01 *M* $HClO_4$ + 0.01 *M* TNBSA	0.5231	−0.3903	−0.3799	0.999
Inorganic acids					
1	0.01 *M* NSA + 0.01 *M* $HClO_4$	0.5024	0.4325	0.4404	0.999
2	0.01 *M* NSA + 0.01 *M* H_2SO_4	0.5068	0.1701	0.1609	0.997
3	0.01 *M* NSA + 0.01 *M* H_3PO_4	0.4933	0.1668	0.1601	0.999
4	0.01 *M* NSA + 0.01 *M* $NaClO_4$	0.4969	−0.2143	−0.2186	0.999

* The natural logarithmic values of k' for PRC where N–CH_3 is absent ($N = 0$).

** r = Correlation coefficient obtained from regression analysis.

observed chromatographic behavior of the analyte components. A chromatogram showing adequate resolution of a mixture of nine imminium dye components is presented in Fig. 11.

The chromatographic optimization is often facilitated by the addition of NSA to the mobile phase to form a binary system with another good acid electrolyte. For the series of three acetic acids coupled individually with NSA (Table II), the retention of the dye samples tends to increase with increasing pK_a values of the acetic acids concerned (Table II).

Comparisons of the results (Figs. 3–5, 7–9) obtained with column A with those of column B show distinct differences. The octadecyl bonded stationary phase in column A is less retentive and gives less symmetric peaks than that in column B. While the surface coverage of the column A packings by octadecylsilylation is nearly complete (density of bonded groups, 4 μmol/m^2), that of column B is relatively low (density of bonded groups, 1.53 μmol/m^2). In practice, it is preferable to use column B for quantitative analytical work in order to attain accurate and reliable quantification of the dye samples. It has not been established whether the shape, spherical (A) *versus*

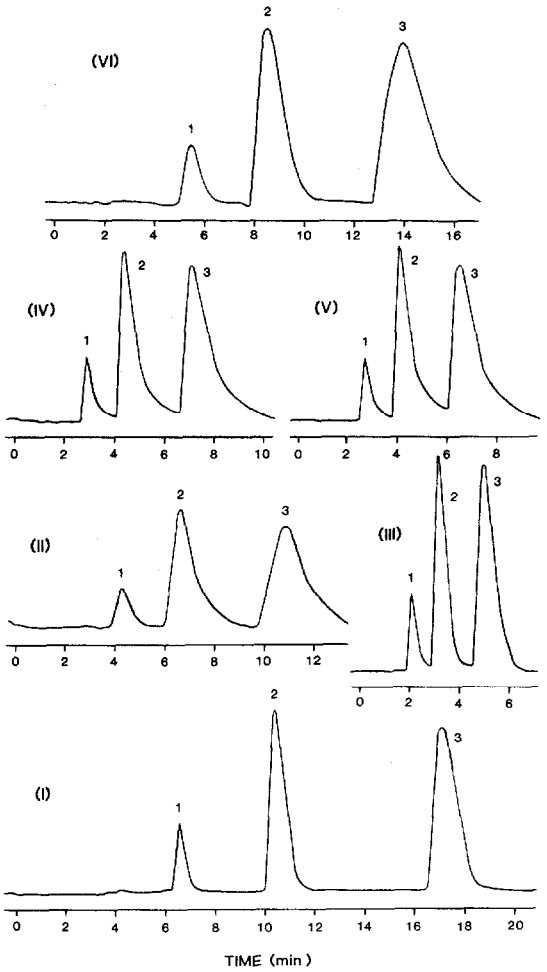

Fig. 10. Effect of various added electrolyte reagents on the chromatographic behavior of some commercial MV samples. Peak identities: 1 = MV(T); 2 = MV; 3 = CV. Mobile phase: acetonitrile–water (70:30) containing 0.01 *M* $HClO_4$ + 0.01 *M* NSA (I), 0.01 *M* $HClO_4$ + 0.01 *M* MSA (II), 0.01 *M* $HClO_4$ + 0.01 *M* TSA (III), 0.01 *M* H_2SO_4 + 0.01 *M* NSA (IV), 0.01 *M* H_3PO_4 + 0.01 *M* NSA (V), 0.01 *M* CCl_3COOH + 0.01 *M* NSA (VI). Flow-rate under isocratic elution: 3 ml/min (column B). See Table I for abbreviations of reagent names.

irregular (B), of the column packings has any bearing on the difference in peak characteristics.

A calibration study was conducted for the application of the HPLC method to the quantitative analysis of the triphenylmethane dyes. The detector response for each analyte was found to vary linearly with the amount of the sample injected. In all cases examined, the linear relationship holds over the range 1 ng–5 μg. The minimum detectable concentration is in the neighbourhood of 0.1 μg/ml. The relative standard deviations of triplicate determinations had values ranging from 2.1 to 8.3 %. Analysis of environmental samples including those of aqueous and organic tissue origins can

TABLE II

THE EFFECT OF THE TYPE OF ADDED ELECTROLYTE REAGENT ON THE CAPACITY FACTOR, k', OF SOME TRIPHENYLMETHANE DYES

Flow-rates for columns A and B were 2 ml/min and 3 ml/min respectively (iscoratic elution); the eluent pH was adjusted to 3.00 with K_2HPO_4 in each case. Data were compared by varying one of the added reagents in the binary electrolyte systems; TCA = CCl_3COOH; other abbreviations used are as in Table I. NA = Not available; pK_a values are listed only for the acids under comparison.

No.	*Mobile phase (acetonitrile–water, 70:30)*	pK_a	*Capacity factor, k'*									
			PRC	*BF*	*MV(T)*	*MV*	*MG*	*CV*	*MeG*	*BG*	*VB*	*EV*
Column A:												
Acetic acids												
1	0.01 *M* NSA + 0.1 *M* CH_3COOH	4.75	0.49	0.60	4.13	6.52	7.81	11.5	13.2	14.4	15.7	52.4
2	0.01 *M* NSA + 0.1 *M* CCl_3COOH	0.66	0.39	0.48	2.67	4.13	4.95	6.86	8.09	9.67	9.99	25.4
3	0.01 *M* NSA + 0.1 *M* CF_3COOH	0.23	0.24	0.33	1.86	3.00	3.40	5.08	5.93	6.41	6.78	16.6
Alkanesulfonic acids												
1	0.1 *M* $HClO_4$ + 0.01 *M* MSA	−0.60	0.19	0.21	1.17	1.83	2.04	2.81	3.77	4.31	4.94	6.97
2	0.1 *M* $HClO_4$ + 0.01 *M* PSA	NA	0.22	0.27	1.38	2.13	2.39	3.38	4.46	5.00	5.58	8.13
3	0.1 *M* $HClO_4$ + 0.01 *M* OSA	NA	0.36	0.43	2.11	3.05	3.10	4.71	5.39	6.63	7.62	11.1
Aromatic sulfonic acids												
1	0.01 *M* $HClO_4$ + 0.01 *M* NSA	0.57	0.34	0.53	2.57	4.14	5.16	6.71	9.13	11.3	9.14	30.6
2	0.01 *M* $HClO_4$ + 0.01 *M* BSA	2.55	0.44	0.56	3.14	6.01	6.19	9.21	9.76	10.6	11.3	39.6
3	0.01 *M* $HClO_4$ + 0.01 *M* TSA	NA	0.32	0.40	2.43	3.86	4.66	6.43	7.21	8.09	7.96	25.7
4	0.01 *M* $HClO_4$ + 0.01 *M* TNBSA	NA	0.42	0.62	2.56	3.86	4.48	6.29	7.14	8.45	7.73	27.6
5	0.01 *M* TCA + 0.01 *M* NSA	0.57	0.37	0.54	2.71	4.43	5.16	7.29	8.20	9.35	9.89	31.6
6	0.01 *M* TCA + 0.01 *M* TSA	NA	0.33	0.41	2.70	4.43	6.07	7.43	8.17	9.91	9.78	33.9
7	0.01 *M* TCA + 0.01 *M* TNBSA	NA	0.41	0.52	3.14	4.86	7.00	8.29	8.99	10.3	9.47	29.6
Column B:												
Inorganic acids												
1	0.01 *M* NSA + 0.01 *M* $HClO_4$	−8.0	1.55	2.03	20.7	33.3	31.4	55.7	59.7	53.0	51.3	143
2	0.01 *M* NSA + 0.01 *M* H_2SO_4	−3.0	1.17	1.30	8.67	13.0	14.2	22.7	30.4	19.7	19.7	48.3
3	0.01 *M* NSA + 0.01 *M* H_3PO_4	2.1	1.17	1.23	8.33	13.0	13.0	20.7	27.6	17.7	16.7	42.3

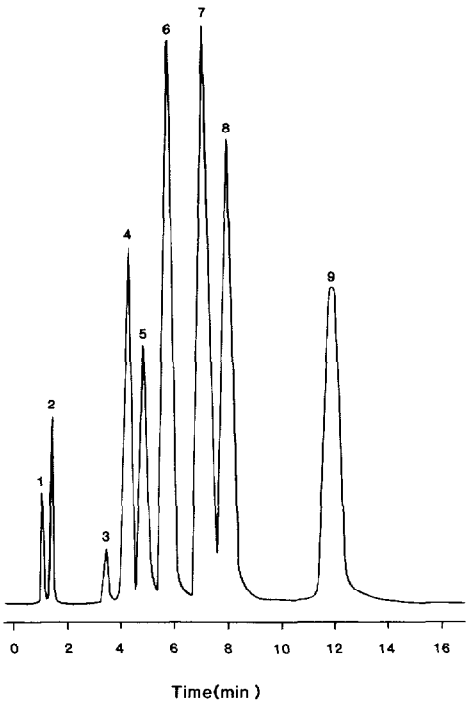

Fig. 11. HPLC separation of a mixture containing selected triphenylmethane dyes. Components: 1 = PRC; 2 = BF; 3 = MV(T); 4 = MG; 5 = MV; 6 = BG; 7 = CV; 8 = MeG; 9 = EV. HPLC conditions: acetonitrile–water (90:10) containing 0.01 *M* $HClO_4$ and 0.01 *M* NSA; flow-rate, 2 ml/min under isocratic elution through column B.

be donc with high reproducibility without recourse to tedious clean-up steps. This is partly attributed to the intrinsic spectrophotometric property of the dyes which offers an advantage in the provision of detection specificity. The analytical procedure involves preconcentration and purification of the spiked samples by chromatography through separate columns of organic polymeric resins (XAD-type) and of ion-exchange resins (cationic) followed by liquid–liquid ion pair extractions. The method is similar to that reported previously for MG and related compounds of environmental importance[6]. A detailed description of the procedure will be elaborated in a separate paper.

It is concluded that the HPLC method developed here permits the separation and quantitative measurement of the quinoidal imminium compounds derived from triphenylmethanes. In the light of the parallelism between the HPLC results for the quaternary ammonium and quinoidal imminium salts of large molecular size, the method may find application in the analysis of other high-molecular-weight imminium compounds.

REFERENCES

1 T. Biclicky and M. Novak, *Arch. Dermatol.*, 100 (1969) 540.
2 D. Y. Fung and R. D. Miller, *Appl. Microbiol.*, 25 (1973) 793.
3 U. Lieder, *Naturwissenschaften*, 48 (1961) 437.

4 J. Dobrecky and R. C. Bonino, *Rev. Asoc. Bioquim. Argent.*, 36 (1971) 143.
5 Aldrich Chemicals Company, personal communication.
6 S. L. Abidi, *183rd National Meeting of the American Chemical Society, Las Vegas, NV, March 29–April 2, 1982*, Abstracts Book ANAL 25.
7 S. L. Abidi, *J. Chromatogr.*, 213 (1981) 463.
8 Cs. Horváth, W. Melander and I. Molnár, *Anal. Chem.*, 49 (1977) 142.
9 R. K. Iler, *The Chemistry of Silica*, Wiley, New York, 1978, p. 660.