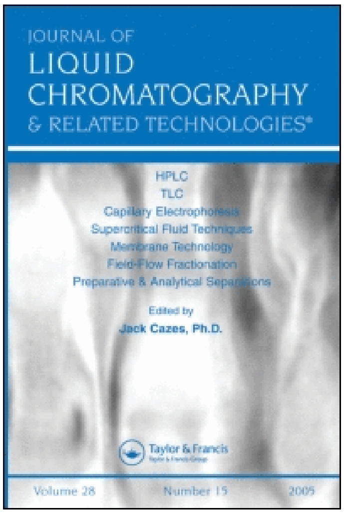


Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

RP-HPLC Separation of Highly Charged Quaternary Ammonium Salts

F. P. Schmidtchen[a]; H. Oswald[a]
[a] Lehrstuhl für Organic Chemie und Biochemie der TU München, Lichtenbergstrasse 4, Federal Republic of Germany

To cite this Article Schmidtchen, F. P. and Oswald, H.(1986) 'RP-HPLC Separation of Highly Charged Quaternary Ammonium Salts', Journal of Liquid Chromatography & Related Technologies, 9: 5, 993 — 1002

To link to this Article: DOI: 10.1080/01483918608076685

URL: http://dx.doi.org/10.1080/01483918608076685

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(5), 993-1002 (1986)

RP-HPLC SEPARATION OF HIGHLY CHARGED QUATERNARY AMMONIUM SALTS

F. P. Schmidtchen and H. Oswald
Lehrstuhl für Organic Chemie
und Biochemie der TU München
8046 Garching
Lichtenbergstrasse 4
Federal Republic of Germany

ABSTRACT

The reverse phase HPLC separation of polycationic viologenes 1 - 4 and macrotricyclic quaternary ammonium salts 5 - 8 using ion pair conditions is described. Whereas the compounds 1 - 4 could be analysed on any of 4 stationary RP-18 phases tested, the cage compounds 5 - 8 were much more sensitive to the source of the matrix material. Optimal separation conditions for the latter up to eightfold positively charged macrocycles employ sodium perchlorate in acidic aqueous methanol using Nucleosil RP-18 or Lichrospher CH-18 columns.

INTRODUCTION

In the course of our work directed towards the synthesis of artificial anion receptors we developed the concept of polycationic polymacrocyclic cage compounds 5 - 8 (1). The synthesis, characterisation and control of purity of these multiply charged com-

0148-3919/86/0905-0993$3.50/0

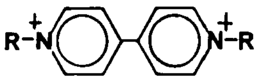
pounds requires quick and reliable analytical and preparative separation methods. Initial attempts to make use of the most prominent property of the artificial receptors and the intermediates in their synthesis, their permanent positive charge, in ion exchange- or electrophoretic analysis failed to give acceptable results due to poor resolution. Ion pair chromatography on reverse phases in contrast should in addition to its dependence on charge be sensitive to the polarizability and hydrophobicity of the compound and thus appears more promising for the separation of structually related molecules. We now report on our experience to separate hydrophobic viologene-type compounds 1 - 4 and more hydrophilic polyammonium salts 5 - 8 via ion pair RP-HPLC.

EXPERIMENTAL

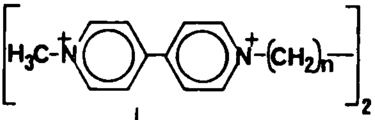
The viologenes 1 - 4 were either commercially avaible (1, 2,Sigma) or were obtained according to known methods (5). The synthesis of macrotricyclic ammonium salts 5 - 8 has been described (6,7). Chromatographic analyses were performed using a Merck-Hitachi HPLC solvent delivery system (model 655A-11 pump attached to a 655A-71 low pressure gradient device) connected to a Rheodyne 7125 injector and a Knauer model 9700 UV-detector. The columns (250 x 4mm) were purchased: Hyperchrome Nucleosil 100 RP-18, 7 μm (Bischoff), Hibar Lichrospher 100 CH 18/2, 5 μm (Merck), Ultrasphere Ion pair C-18 IP, 5 μm (Beckman). Standard analysis before and after polycation separation confirmed that the columns experienced no significant deterioration in the process. All chemicals were reagent grade and used without further purification.

RESULTS AND DISCUSSION

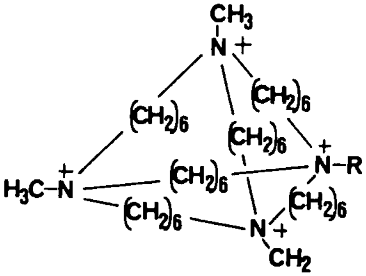
Although RP-HPLC deserves its reputation of being fast, reliable and reproducible, the separation of basic or positively charged

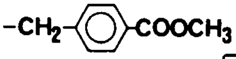
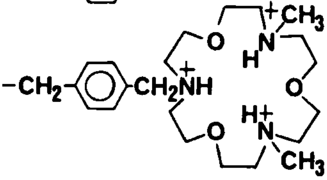
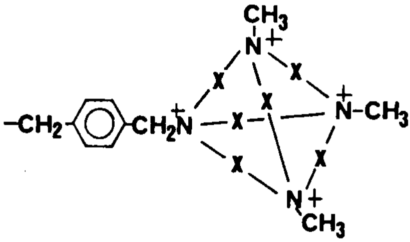


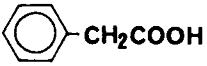
	R
1	$-CH_3$
2	$-CH_2C_6H_5$



	n
3	3
4	5



	R
5	
6	
7	 X= $-(CH_2)_6-$
8	X= $-(CH_2)_8-$



9

compounds presents problems associated with peak shape and peak broadening which frequently obstructs quantification of the analysis. The reason is attributed to the presence of anionic silanol groups in the SiO_2 bead matrix that have escaped the chemical modification process. Nevertheless quite acceptable separations of monoammonium salts can be achieved if the mobile phase contains tertiary ammonim salts in addition to anionic ion pair reagents (2). These salts are supposed to deactivate the silanol groups so that they do not interfere with the ion pair distribution between mobile and stationary phases (3).

This system consisting of triethylammonium formate buffer (pH 3.5 in water), sodium heptanesulfonate and varying amounts of methanol taking Lichrosorb RP-18 as the stationary phase sufficed to give satisfactory separations of the polycationic hydrophobic viologenes 1 - 4 (Fig. 1) but failed totally with the cage compounds 5 - 8. There was no way to improve the separation in this system by systematic variation of the concentrations of organic modifier, ammonium salt additive or ion pair reagent.

We felt that the poor shape and excessive broadening of the peaks obtained in the latter case could result form slow mass transfer attributable to the peculiar properties of the stationary phase used as well as from a specific interaction of the hydrophobic heptanesulfonate ion pair reagent with the macrotricycles. It is well established that the cationic cage compounds bind anions and hydrophobic molecules in aqueous solution by inclusion complexation (1b,4). The kinetics of these processes may be slow enough to lead to peak broadening in the chromatographic analysis. Thus it seemed necessary to vary the eluent composition in a way to obviate the limitation from slow counterion exchange kinetics while retaining the hydrophobic character of the substrate-counterion complexes.

In addition CPK-model inspection shows that the longest molecular diameter of 8 is about 40 A. Consequently association of counter

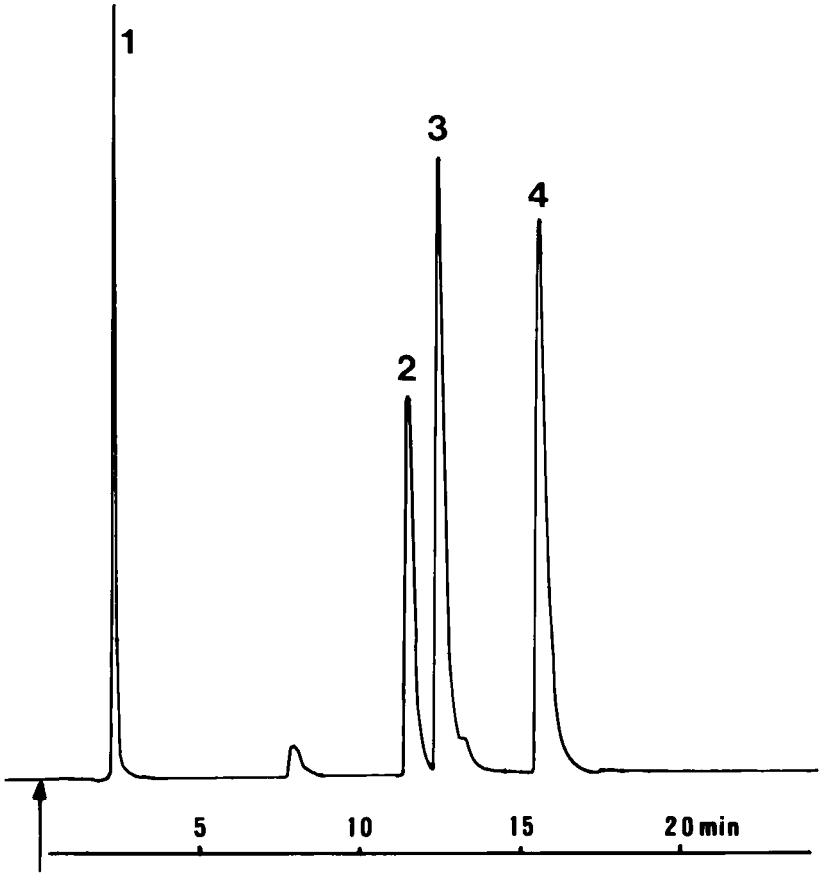


FIGURE 1: Separation of viologenes 1 - 4 on Nucleosil RP 18; Flow 1.0 ml/min; Gradient: A/B = 83/17 → A/B = 50/50 (9 min) hold for 20 min, return to starting conditions; A = 40% CH_3OH, 30mM triethylammonium formate buffer pH 3.50, 10mM Heptanesulfonate; B = 70% CH_3OH, 30mM triethylammonium formate buffer pH 3.50, 10mM Heptanesulfonate; UV_{254}-detection.

ions to the eightfold positively charged ion may easily yield complexes comparable in size to the average pore diameter of the stationary phase matrix. One should infer therefore that the separation of these compounds is sensitive to the uniformity of the chemically bonded layer and its accessability within the silica structure.

Initial attempts to use octyl-(RP-8) or cyanopropyl (CN) coated silica as stationary phases were soon abandoned, because the retention of the hydrophilic quaternary ammonium compounds 5 - 8 was too poor. Instead we focused our studies on octadecyl (RP-18) coated silica and compared commercial columns of identical dimensions but from 3 different manufacturers knowing that retention data, peak shape and column performance may heavily depend on the source of the matrix material although nominally the same material is used. The columns are packed wih 5 or 7 μm spherical particles of 100 A average pore size (where specified, see experimental part) the surfaces of which are totally covered by hydrocarbon layer or specifically designed for ion pair analysis.

To optimize the mobile phase we decided to vary the concentration of an organic modifier (solvent: methanol or acetonitrile) by a gradient device holding the concentrations of buffer and ion pair reagent constant. The selection of the latter additive was guided by the notion that small but still lipophilic counter anions to the highly positively charged ammonium salt had to be used in order to avoid peak broadening due to slow complex formation kinetics. Most of the usual halide- or oxoanion salts of the compounds 5 - 8 are highly water soluble and therefore not likely to be retarded in an ion pair chromatographic system. However, chaotropic anions like perchlorate or tetrafluoborate precipitate these compounds from aqueous solutions these salts being readily soluble in dipolar solvents like acetonitrile or nitromethane. Pilot experiments taking sodium perchlorate as an ion pair additive in the mobile phase indeed furnished acceptable retention

Table 1: Retention data (k -values) of polyammonium compounds 1 - 8 under standardized chromatographic conditons: stationary phase: 250 x 4 mm RP-18 column; linear gradient profile 100% A - 100% B (30 min), hold 10 min, return to A; A: 10v/v% methanol, 30mM $NaClO_4$, 30mM HCOOH; B: 60v/v% methanol, 30mM $NaClO_4$, 30mM HCOOH; Flow: 1.0 ml/min; Detection: UV_{254}

	Nucleosil	Lichrospher	Ultrasphere
1	0.1	0.1	0.1
2	7.7	8.6	9.08
3	1.0	0.8	5.0
4	5.7	6.4	6.3
5	9.3	9.9	10.4
6	6.7	7.3	8.0
7	8.8	9.8	8.0
8	11.4	13.7	14.7

values with the hydrophilic ammonium macrocycles 5 - 8 on RP-18 stationary phases. The optimum concentration of perchlorate was 30 mM considering the opposing influences of its concentration on sample retention, requirement of organic modifier, viscosity of the eluent and cost per analysis.

The retention data of the multiply charged ammonium compounds 1-8 under standardized chromatographic conditions using perchlorate counterion and methanol as an organic modifier are given in Table 1.

These figures reveal that a variety of polycationic ammonium compounds is indeed separable by means of ion pair HPLC using perchlorate as a counter anion. The columns tested, however, show great differences in their utility in this process. Although the sequence of elution of the compounds is qualitatively the same for any of the columns major deviatiations in their relative retention are observed. Apparently the overall hydrophobicity of the compound dominates its retention behaviour rather than the number of charges as can be deduced from the data of the viologenes 1 - 4 and the macrocycles 5 - 8. In general the capacity factors increase with the same compound being analyzed on the Nucleosil-, Lichrospher or Ultrasphere columns.

Differences in the number of theoretical plates and peak shape are even more pronounced. Qualitatively the nucleosil and Lichrospher columns are very much alike concerning their effects on peak broadening and shape whereas the Ultrasphere column is much worse in this respect, particularly with the larger compounds 7 and 8. A quantitative evaluation of the change in peak shape is given in Table 2.

Obviously any column experiences a decrease in n_{th} and an enhancement of peak deformation but the excessive effect observed with Ultrasphere makes even peak identification rather difficult. The

Table 2: Comparison of theoretical plate numbers n_{th} and asymmetry factors ASF for an acidic compound phenylaceteate 9 and the strongly basic octaammonium salt 8 using isocratic conditions (9: 35v/v% CH_3OH, $NaClO_4$, HCOOH 30mM each; 8: 48% CH_3OH, $NaClO_4$, HCOOH 30mM each; Flow 1.0 ml/min)

column/particle size	9			8		
	k	n_{th}	ASF	k	n_{th}	ASF
Nucleosil/7/um	4.8	8500	1.2	4.4	710	3.0
Lichrospher/5/um	6.1	17500	1.05	3.3	1160	4.6
Ultrasphere/5/um	7.5	21000	1.35	21.7	240	4.1

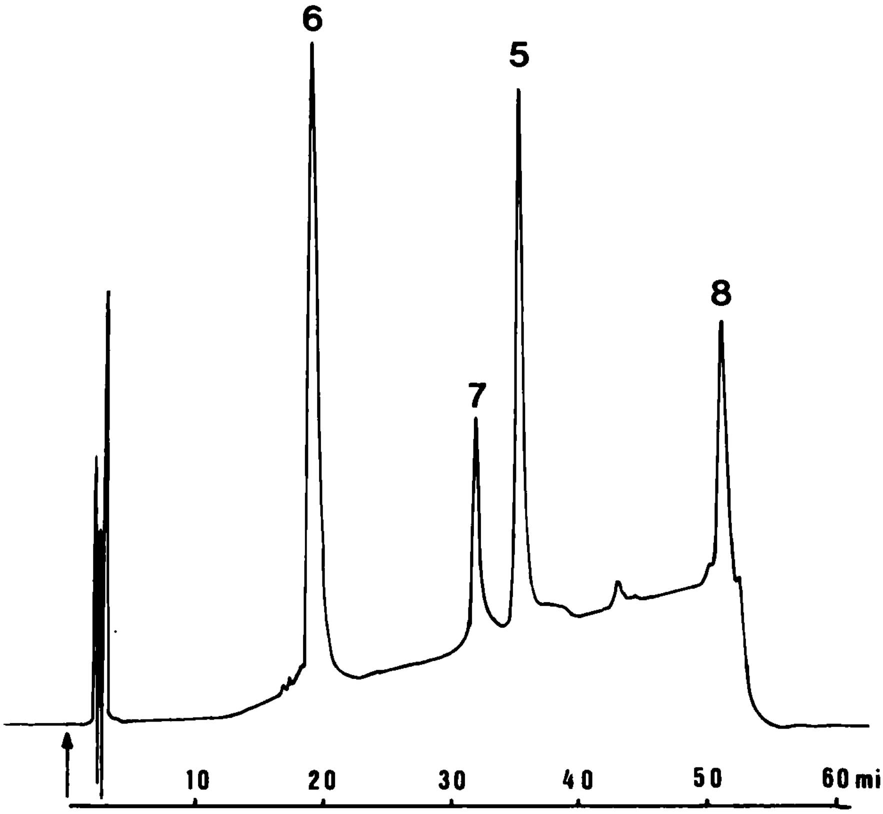


FIGURE 2: Separation of macrotricyclic ammonium salts 5 - 8 on Lichrospher 100 CH-18; Flow 1.0 ml/min; Gradient: 100% A → 100% B (45 min); A: 20% v/v CH_3OH, 30mM HCOOH, 30mM $NaClO_4$; B: 50% CH_3OH, 30mM HCOOH, 30mM $NaClO_4$; UV-detection.

diminution of resolving power in the former cases affects the separability of cationic compounds (Fig. 2) only marginally, because the great sensitivity of the retention of polycationic compounds towards change in concentrations of organic modifier, buffer or ion pair reagent offers sufficient degrees of freedom to assess chromatographic conditions for the separations of any two sample components.

ACKNOWLEDGEMENT

We are grateful for the financial support of this work by Deutsche Forschungsgemeinschaft and to Mrs. M. Pech for the gift of samples of compounds 3 and 4.

REFERENCES

1) a)Schmidtchen, F.P., Angew. Chem. Int. Ed. Engl. 16, 720 (1977)
b)Schmidtchen, F.P., Chem. Ber. 114, 597 (1981); ibid. 117, 1287 (1984)

2) Wahlund, K.G., Sokolowski, A., J. Chromatog. 151, 299 (1978); ibid. 189, 299 (1980)

3) cf. Reynolds, D.L., Riley, C.M., Sternson, L.A., Repta, A.J., J.Pharm. Biomed. Anal. 1, 339, 347 (1983)

4) Schmidtchen, F.P., Müller, G., J. Chem. Soc. Chem. Commun. 1984, 1115

5) a)Hünig, S., Geuder, W., Suchy, A., Angew. Chem. Int. Gd. Engl. 22 489 (1983);
b)Hünig, S., Berneth, H., Top. Curr. Chem. 92, 1 (1980)

6) Schmidtchen, F.P., Tetrahedron Lett. 1984

7) Schmidtchen, F.P., submitted