Journal of Chromatography, 256 (1983) 39–48
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,385

LIQUID CHROMATOGRAPHY OF PHENOLS ON AN 8-QUINOLINOL SILICA GEL–IRON(III) STATIONARY PHASE

G. J. SHAHWAN* and J. R. JEZOREK*
Department of Chemistry, University of North Carolina at Greensboro, Greensboro, NC 27412 (U.S.A.)
(Received September 21st, 1982)

SUMMARY

Iron(III)-loaded 8-quinolinol silica gel (QSG) is shown to be an effective stationary phase for the high-performance liquid chromatographic separation of phenols, including EPA Priority Pollutant species. The QSG itself exhibits significant reversed-phase and hydrogen-bonding interactions with sample compounds, but the presence of the iron(III) on the QSG substantially increases both retention and resolution. Very good column stability is observed, with no bleeding of chelated metal ion. Therefore, the presence of iron(III) in the mobile phase is not necessary. Preferred column conditions are a water–acetonitrile mobile phase buffered at pH 4 with acetic acid–sodium acetate. A study of retention volumes as a function of pH suggests that acetate species competitively interact with stationary phase sites.

INTRODUCTION

The use of metal-loaded stationary phases to enhance retention and selectivity in chromatography is a relatively well-established technique. Several different metal ions have been used to obtain selectivity for various types of solutes. The gas (GC) and thin-layer (TLC) chromatographic separation of unsaturated hydrocarbons and aromatic compounds using silver(I)-impregnated stationary phases is an early example[1]. This so-called argentation technique has also been applied in the liquid chromatographic area as exemplified by the work of Aigner *et al.*[2]. Latterell and Walton[3] used a nickel(II)-loaded cation-exchange resin to separate amines, while a study of the separation of aromatic amines by Kunzru and Frei[4] employed cadmium(II)-impregnated silica gel as the stationary phase. For the most part, silica gel itself or polystyrene-based ion-exchange resins have been employed as the support for these metal-assisted chromatographic studies.

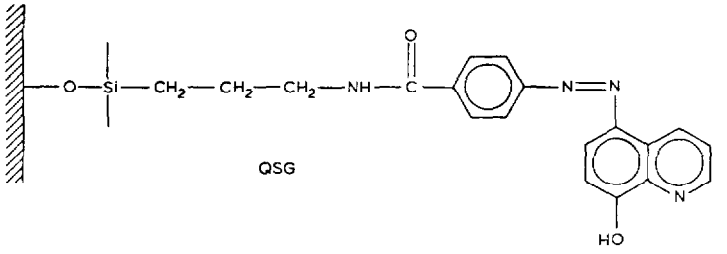
The great utility of this approach is that different metal ions can provide selectivity for different classes of chromatographic samples; and sample components with little or no electron-donating properties are not affected by the presence of the

* Present address: Department of Chemistry, University of New Orleans, New Orleans, LA 70148, U.S.A.

0021-9673/83/0000–0000/$03.00

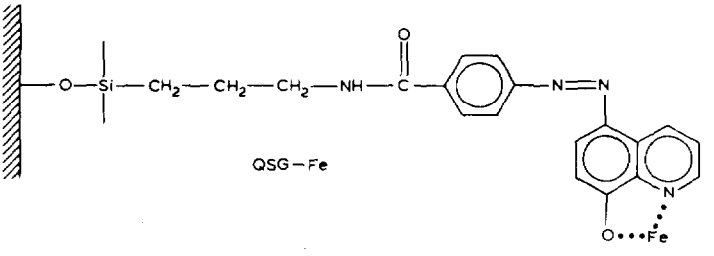
metal ion in the stationary phase. A drawback to the technique is the bleeding of the metal ion from the silica gel or the ion-exchange stationary phases. However, the advent of chelating stationary phases which bind metal ions more strongly has renewed interest in the use of metal ions in liquid chromatography. These chelating materials have substantially reduced the problem of metal bleeding.

Some very interesting work has been recently performed using chelating ligands covalently bonded by silylation reactions to silica supports. For example, Masters and Leyden[5] utilized copper(II)-loaded *n*-propylethylenediamine on controlled-pore glass for the separation of some amino sugars and amino acids, and Cooke *et al.*[6] used this same ligand with cadmium(II) for the separation of sulfa drugs. Chow and Grushka[7] employed copper(II)-loaded diketone- and dithiocarbamate-silica gel to separate a number of aromatic amines.



QSG

Our interest in this area stemmed from the perception that 8-quinolinol silica gel (QSG), an immobilized ligand with which we have had considerable experience[8–10], ought to be a very good support for metal-assisted high-performance liquid chromatography (HPLC). This material binds many metals very tightly and might alleviate or, in some cases, eliminate, bleeding of the ions from the stationary phase, a problem that has remained even in recent work with silica-bound ligands[5,11]. QSG-packed columns can be easily loaded with metal, stripped and re-loaded with a different metal *in situ*, making repacking unnecessary. Therefore, as QSG binds a large variety of metal ions, selectivity for various classes of samples can be effected without changing the support. This material has been shown to have 1:1 stoichiometry with metal ions[10], so that while two coordination sites of the metal are involved with chelation, as shown below for iron-loaded QSG, the remaining ones are merely solvated and remain available for interaction with solute species:



QSG–Fe

Most metal-assisted HPLC studies have involved separations of unsaturated hydrocarbons, amines or amine-substituted species, but, to our knowledge, no study of phenols, an environmentally important class of compounds, has been reported

using this approach and modern LC techniques. It was desirable to see if these compounds were amenable to separation using metal-loaded QSG as the chromatographic stationary phase. Iron(III) was chosen as the metal ion as it has a large formation constant with 8-quinolinol and because it has great affinity for oxygen and should interact readily with the hydroxyl moiety of the phenols.

Chow and Grushka[7] and Cooke *et al.*[6] have discussed the loss of efficiency that may occur because of too strong binding with the metal ion, and we wished to examine this effect in this study. It was also of interest to determine whether the phenols interact significantly with the QSG itself in the absence of the iron(III).

EXPERIMENTAL

Apparatus

Chromatographic studies were performed with a Spectra-Physics SP-8000 gradient liquid chromatograph fitted with a 55-μl tantalum sample loop (Valco, Houston, TX, U.S.A.). The detector was a Schoeffel Model 770 (Kratos, Ramsey, NJ, U.S.A.) operated at 254 or 280 nm. A pneumatic pump Model 26980-4 (Haskel, Burbank, CA, U.S.A.) was used to slurry-pack the columns. Stainless-steel columns were 250 × 4.6 mm I.D. with 2-μm stainless-steel end frits.

Reagents and solutions

All aqueous solutions were prepared with doubly-distilled deionized water. Phenols were obtained from Aldrich or Fisher and used as received. Sample solutions were about $1 \cdot 10^{-4}$ *M* in methanol–water (50:50) or acetonitrile–water (50:50). Mobile phases used ACS grade methanol or acetonitrile initially, but more highly purified solvents in later phases of the work (UV grade acetonitrile, Burdick and Jackson; HPLC grade methanol, J. T. Baker). All samples and eluents were filtered through a 0.45-μm membrane before use. Buffer reagents were ACS grade and were purchased from a variety of suppliers.

The 3-aminopropyltriethoxysilane was obtained from Pierce (Rockford, IL, U.S.A.) and the trimethylchlorosilane from PCR Research Chemicals (Gainesville, FL, U.S.A.). These were stored in a refrigerator until use. Organic solvents used for the preparation of the modified silica gel were ACS grade and were dried over 4-Å molecular sieves. Triethylamine (Eastman) was distilled before use. The *p*-nitrobenzoyl chloride (MC/B) was used as received. All other reagents used in preparation of the bound 8-quinolinol were ACS grade and used without further purification.

Two grades of silica gel were used in this study. For preliminary experiments and pH–response studies TLC grade, HR extra pure (E. Merck) was used. This material has 60-Å pores, a surface area of 550 m^2/g and had an initial size range of 10–50 μm. This range was narrowed as previously described[9]. Examination under 750 × magnification revealed that most particles were in the 30–50 μm range. For most of the chromatographic studies Adsorbosil-LC preparative-grade silica gel was used (Applied Science Labs., State College, PA, U.S.A.). This material had 70-Å pores, a surface area of 480 m^2/g and 10 μm diameter. Microscopic examination confirmed the vendor's specification that 80% of the particles were in the 6–13 μm size range. After preparation of the QSG examination under the microscope revealed that an insignificant amount of fines were formed during the series of reactions so that the integrity of the original size distribution was retained.

Preparation of bound 8-quinolinol

Covalent binding of the 8-quinolinol to the silica gels was based on the methods of Hill[12] and Weetall and co-workers[13–15] to which certain modifications were made. These have been described[9]. After the initial silylation reaction the 10-μm aminopropyl silica gel was extracted with acetone and then methanol in a Soxhlet apparatus to remove adsorbed, unreacted, silane and polymer not covalently attached to the surface. The presence of some of this material was revealed by the yellowish brown residue which remained after evaporating most of the extracting solvents.

At the nitrobenzamide (NBSG) stage of the preparation[9] the residual silanol sites were capped by refluxing the NBSG under nitrogen in 10% trimethylchlorosilane–toluene. The capped material was filtered and washed with dry toluene, methanol and acetone. The rest of the preparation was completed as previously reported[9]. Washing the red QSG with methanol, acetonitrile or an aqueous mixture of either of these yielded a yellow-to-red solution. UV–visible spectroscopic analysis of this solution and of the washings of the intermediate materials in the preparation scheme suggests two possibilities, that *p*-nitrobenzoyl chloride adsorbs on or reacts with –SiOH sites and takes part in subsequent reactions, and/or that the original silylation with 3-aminopropyltriethoxysilane produced polymeric materials or adsorbed species which also eventually yielded the azo-coupled quinolinol. In any case, these adsorbed or weakly bound materials were gradually washed off by the polar organic solvents or aqueous mixtures. Similar behavior with silica-based ion-exchange materials has been observed by other workers[16]. Before use, all columns were washed with methanol–water (50:50) until the effluent was colorless. Loading of iron(III) on the QSG was found to reduce significantly leaching of these colored materials from the column for reasons which are not known at this time.

Metal-uptake capacity of QSG

The amount of 8-quinolinol bound to the surface was determined by copper-uptake studies as previously described[9]. Iron(III)-uptake determinations were also performed using a procedure similar to that for copper(II). The iron was stripped off the QSG with 30 ml of 1 *M* hydrochloric acid, added in several portions. A potassium thiocyanate spot test was used to be sure no further iron was being removed. The amount of iron(III) in this extract was determined by EDTA titration with salicylic acid indicator. Good agreement with the copper-uptake results was obtained. The 8-quinolinol content of the 30–50 μm QSG was 0.19 μmol/m^2 while that of the 10-μm material was 0.37 μmol/m^2.

Column packing and metal loading

Columns using the 30–50 μm QSG were dry-packed[17]. The 10-μm QSG was slurry-packed, according to the method of Manius and Tscherne[18], at 6000 p.s.i. using a Haskel pneumatic pump and methanol solvent. Iron(III) was loaded on the QSG columns *in situ* by slowly pumping 0.01 *M* aqueous iron(III) nitrate through the column until the effluent gave a positive potassium thiocyanate test. Pumping was continued for an additional 30 min to be sure that all sites were loaded. Excess iron(III) was washed off with water to a negative thiocyanate test. An alternative procedure was to batch-load the QSG (4 g) in 100 ml of 0.01 *M* iron(III) nitrate by stirring the mixture for 30 min, filtering and washing thoroughly with water. The

QSG-Fe was jet-black, as compared to the brick red of QSG itself. Periodically during the chromatographic studies the column effluent was tested for iron, always with negative results. In fact, the iron(III) was stripped off one column with 1 *M* hydrochloric acid after 300 h of use and the amount of iron removed was exactly the same as had been loaded on the column. The tenacity with which the QSG holds the iron(III) is a distinct advantage in that no metal ion needs to be added to the mobile phase to compensate for bleeding and very stable column conditions are realized.

Chromatographic procedure

Packed columns were equilibrated for 20–30 min or until a steady detector baseline was achieved. Solvents were degassed for 20 min by bubbling with helium. The column-compartment temperature was 40°C. The 10-μm column void volume was determined to be 1.8 ml. All studies were done at a flow-rate of 1 ml/min.

RESULTS AND DISCUSSION

As metal-assisted chromatography involves Lewis acid–base interactions it was expected that retention of phenols on a QSG-Fe column would be pH dependent. In order to determine a suitable pH region in which to work, separation of a number of phenols was attempted in water–acetonitrile eluent buffered at pH 4 with acetic acid–sodium acetate and at pH 9.5 with ammonia–ammonium nitrate. Very poor resolu-

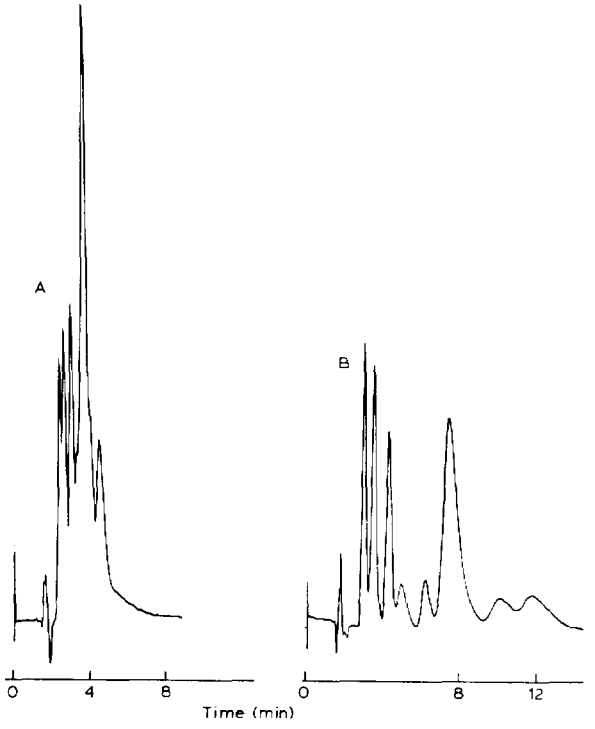


Fig. 1. Isocratic elution of ten phenolic compounds in acetonitrile–0.01 *M* acetate buffer, pH 4.0 (40:60); flow-rate 1 ml/min. A, 10-μm QSG column; B, 10-μm QSG-Fe column.

TABLE I

RETENTION OF PHENOLS ON 10-μm QSG AND QSG-Fe COLUMNS

Mobile phase: 40% acetonitrile–60% 0.01 *M* acetate buffer, pH 4.0. Flow-rate: 1.0 ml/min. Column temperature: 40°C.

Compound	pK_a	*QSG*		*QSG-Fe*	
		V_R *(ml)*	k'	V_R *(ml)*	k'
Phenol	10.0	4.4	0.57	5.7	1.04
p-Cresol	10.2	5.0	0.79	6.8	1.43
o-Chlorophenol	8.5	5.2	0.86	9.6	2.43
2,4-Dimethylphenol	10.5	5.6	1.00	8.2	1.93
m-Nitrophenol	8.4	6.2	1.21	12.0	3.29
o-Nitrophenol	7.2	6.6	1.36	14.8	4.29
4-Chloro-*m*-cresol	–	6.8	1.43	11.6	3.14
p-Nitrophenol	7.1	7.6	1.71	20.4	6.29
2,4-Dichlorophenol	7.8	8.0	1.86	23.2	7.32
2,4,6-Trichlorophenol	7.6	16.0	4.71	58.0	19.7
2,4-Dinitrophenol	4.1	>50.0	>17	>58.0	>19.7
4,6-Dinitro-*o*-cresol	–	>50.0	>17	>58.0	>19.7

tion and severe tailing resulted at the higher pH. Depending on the molecule, partial or complete ionization of the phenol hydrogen occurs at pH 9.5, and the interaction kinetics with the bound iron(III) apparently result in unfavorable mass transfer. All subsequent work was performed in slightly acidic media to maintain the phenols in the neutral state. Another reason for using slightly acidic media is that silica gel itself is known to dissolve under alkaline conditions, the higher the pH the more rapid the dissolution. Ligand-exchange columns run at basic pH values have been shown to deteriorate rather rapidly[5].

In order to investigate the interaction of the phenols with the organic backbone of the stationary phase and the effect of the bound iron on retention and resolution a series of phenols was individually run on both a QSG and QSG-Fe column. Retention volumes (V_R) and capacity factors (k') are given in Table I. At the slightly acidic eluent conditions used all the samples exhibited some retention on the QSG column. The presence of the iron(III), however, significantly increased k' for all solutes tested. Under these same elution conditions the effect of the iron can be seen qualitatively from chromatograms of a mixture of phenols on the two columns (Fig. 1). It is clear that at pH 4 in water–acetonitrile medium both retention and resolution are significantly enhanced by the bound iron(III). Therefore, even though most phenols are not ionized at this pH, interaction of the open (solvated) coordination sites of the metal ion with the phenol does occur, probably with the unshared electrons on the phenol oxygen atom. It was found that peak asymmetry factors on the QSG-Fe column were somewhat larger than on the QSG itself. The presence of the iron(III) evidently results in some mass transfer decrease relative to the QSG column, producing slight tailing. However, the increase in resolution more than compensates for the loss in efficiency, as has been seen before for other metal-loaded systems[7].

Even though it is not our intent to provide a definitive discussion of the phenol–stationary phase interactions, some consideration of this topic seems in order. The

π–π interaction of aromatic species with polystyrene–divinylbenzene resins is well known, and a reversed-phase type interaction of neutral organic species with a silica-based cation-exchange resin has also been reported[19]. The structure of the QSG suggests at least three types of interactions with phenolic compounds: hydrogen-bonding, π–π and dispersion interactions. Hydrogen bonding can occur at the –OH group of the quinolinol moiety, and less strongly at the carboxyl and azo groups. Of course, hydrogen-bonding and dispersion interactions are also possible with eluent components, and the total picture is probably quite complicated. Nevertheless, some relationships between the structure of particular phenols and retention can be noted. For example, the presence of electron withdrawing substituents in the *ortho-* and *para*-positions of the phenol (dinitro- and trichlorophenol) results in substantially increased retention relative to phenol itself. These substituents decrease the electron density in the aromatic ring, and hence the π–π interactions. However they also decrease the electron density on the phenolic oxygen permitting stronger hydrogen bonding by the phenolic proton. The substantially greater retention exhibited by these compounds suggests that hydrogen bonding is a major interaction with the stationary phase.

Methyl-substituted phenol might be expected to have less retention than phenol itself, as a result of electron donation by the methyl group. However, while the phenolic proton is less strongly hydrogen bonding in this situation, the phenolic oxygen is more so. The increased electron density on this atom will increase hydrogen bonding with the proton of the quinolinol moiety of the QSG stationary phase so that these interactions may not decrease much, if at all. Furthermore, the presence of the methyl group(s) results in greater electron density on the aromatic ring and hence π–π interactions, as well as dispersion interactions, are enhanced. Indeed, *p*-cresol and 2,4-dimethylphenol are more strongly retained than phenol itself.

When iron(III) is bound between the nitrogen and oxygen atoms of the quinoline electron density is withdrawn from that aromatic system and decreased π–π bonding with solute species results. Hydrogen bonding at the quinoline is also precluded. And yet, as already noted, the retention of all compounds tested was substantially greater on the QSG-Fe column than on the column without iron. This means that there must be significant interaction of the phenolic oxygen atom with the bound

TABLE II

RETENTION VOLUME AS A FUNCTION OF ORGANIC SOLVENT CONCENTRATION

Mobile phase pH, 5.2; 30–50 μm QSG-Fe column; flow-rate 1 ml/min.

Compound	*Retention volume (ml)*					
	Methanol–0.01 M acetate buffer			*Acetonitrile–0.01 M acetate buffer*		
	40:60	*50:50*	*60:40*	*30:70*	*40:60*	*50:50*
Phenol	5.5	4.8	4.5	6.2	5.2	4.4
p-Cresol	8.2	5.8	4.5	7.2	5.2	4.4
o-Chlorophenol	16.5	8.4	6.6	10.8	7.2	6.5
p-Nitrophenol	32.4	17.3	12.8	19.2	12.4	12.0

TABLE III

EFFECT OF pH ON RETENTION VOLUME IN NITRIC AND ACETIC ACID MOBILE PHASES

Eluent, methanol–aqueous acid (50:50); 30–50 μm QSG-Fe column; flow-rate 1 ml/min; column temperature 40°C.

Compound	*Retention volume (ml)*					
	Acetic acid			*Nitric acid*		
	pH 3.0	*pH 3.3*	*pH 4.3*	*pH 3.0*	*pH 3.3*	*pH 3.8*
Phenol	4.8	5.0	5.8	5.0	4.8	5.2
p-Cresol	5.6	6.2	7.0	6.0	6.0	6.8
o-Chlorophenol	7.0	7.2	11.0	8.4	9.0	10.4
p-Nitrophenol	10.8	11.2	25.4	16.8	18.8	24.0

iron(III). Interaction with the QSG-Fe stationary phase even results in a reversal of two pairs of solutes. On the QSG column *o*-chlorophenol precedes 2,4-dimethylphenol and *m*-nitrophenol elutes before 4-chloro-*m*-cresol. On QSG-Fe, a reversal of the members of each pair occurs (Table I). This situation probably results from the complicated interplay of the several types of interactions mentioned above.

That substantial reversed-phase interactions take place between the neutral phenols and the QSG-Fe support is shown by the effect of methanol or acetonitrile concentration in the eluent at constant pH. For both solvents significant reduction in retention occurs as organic solvent concentration in the mobile phase increases (Table II). Greater solvation of the organic phenols by the organic modifier and/or greater solvation of the backbone of the QSG-Fe support causes a diminution of the three types of interactions discussed above, as well as reversed-phase hydrophobic interactions.

The situation is further complicated by the nature of the pH-control species present in the eluent. By using nitric acid in the mobile phase the effect of pH, essentially uncomplicated by other solvation or complexation factors, was obtained for four representative phenols. The expected increase in retention with increase in pH was indeed found (Table III), as was a correlation with phenol pK_a (Table I). The higher the pH, and the greater the acidity of the phenol, the greater the retention, as the leaving (hydrogen bonding) tendency of the phenol hydrogen increases. The relationship with pK_a is not perfect, however, as the weaker acid *p*-cresol is more strongly

TABLE IV

EFFECT OF pH ON RETENTION VOLUME

Eluent, methanol–0.01 *M* aqueous solution (50:50); other conditions same as for Table III.

Compound	*Retention volume (ml)*						
	Acetate system			*Ammonium acetate*			
	pH 4.0	*pH 5.2*	*pH 6.2*	*pH 3.8*	*pH 4.5*	*pH 5.0*	*pH 5.6*
Phenol	4.8	4.8	5.7	6.0	4.8	5.0	5.2
p-Cresol	5.9	5.8	6.8	7.2	5.7	6.0	6.0
o-Chlorophenol	8.0	8.4	11.5	10.0	8.2	9.3	10.0
p-Nitrophenol	15.2	17.3	26.7	19.1	16.4	20.5	22.8

retained than phenol, due to the substituent effects discussed earlier. If pH control is effected with acetic rather than nitric acid, retention volume at a given pH decreases relative to the nitric acid system (Table III), especially for the more acidic phenols. Several factors may be at work here. Molecular acetic acid and the acetate ion may competitively interact with hydrogen-bonding sites on the QSG-Fe and the phenols themselves. Competition from acetic acid molecules predominates at pH values (3.0 and 3.3) well below the pK_a of acetic acid (4.75). At pH 4.3, where significant numbers of acetate anions are also present, competitive hydrogen bonding and complexation with iron(III) coordination sites may also occur. This complexation effect is minimal for the nitrate ion, however, the formation constant with iron(III) being $10^{3.38}$ $(mol/l)^{-1}$ for acetate and $10^{0.76}$ $(mol/l)^{-1}$ for nitrate[20]. That these competitive interactions are much larger for the acetic acid system than for the nitric acid is further demonstrated by the very similar retention volumes for a given solute at pH 3.8 in the nitric acid, at 4.3 in the acetic acid and at 6.2 in the 0.01 *M* acetate system. In fact, the results obtained with the ammonium acetate system (Table IV) clearly show competitive interactions at work, as a minimum in V_R results on going from pH 3.8 to 5.6. Retention first decreases with pH as acetate competition increases and levels off, then increases as the phenol-hydrogen leaving tendency increases, permitting increased interaction with iron(III) and QSG-Fe hydrogen-bonding sites.

In order to accommodate the several kinds of phenol–stationary phase interactions which are apparently involved and the fairly wide range of phenol pK_a values, it was necessary to use a gradient elution procedure which increased the amount of organic modifier (acetonitrile) in the mobile phase while maintaining a constant pH. The buffer concentration was also held constant at 0.01 *M*. Therefore, assuming that the pH-dependent interactions remain essentially constant with changing concentra-

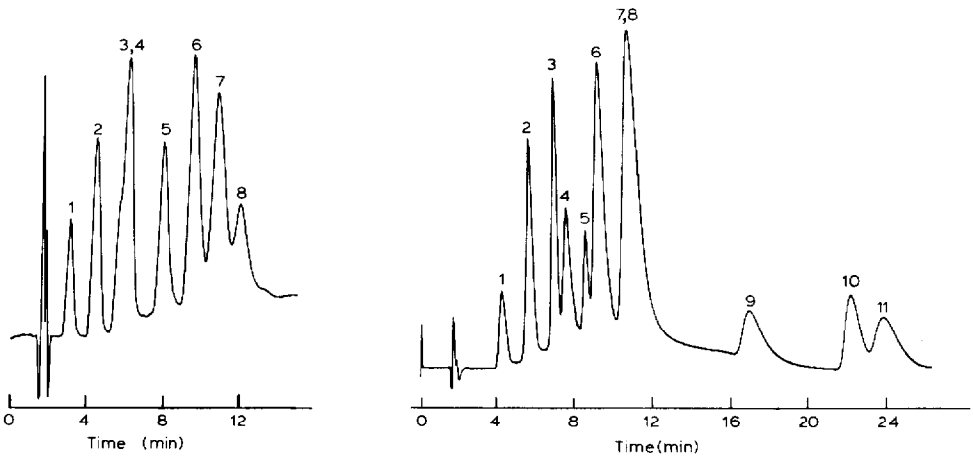


Fig. 2. Chromatogram of a mixture of eight phenols on a 10-μm QSG-Fe column. Mobile phase: acetonitrile–0.01 *M* acetate buffer, pH 4.0; linear gradient from 20 to 70% acetonitrile in 25 min. Peaks: 1 = hydroquinone; 2 = phenol; 3 = *o*-cresol; 4 = *o*-methoxyphenol; 5 = *o*-chlorophenol; 6 = *o*-nitrophenol; 7 = *p*-nitrophenol; 8 = *p*-nitro-*o*-cresol.

Fig. 3. Chromatogram of a mixture of eleven EPA phenolic compounds on a 10-μm QSG-Fe column. Mobile phase: acetonitrile–0.01 *M* acetate buffer, pH 4.0; flow-rate 1 ml/min; linear gradient from 20 to 90% acetonitrile in 30 min. Peaks: 1 = phenol; 2 = *p*-cresol; 3 = 2,4-dimethylphenol; 4 = *o*-chlorophenol; 5 = 4-chloro-*m*-cresol; 6 = *o*-nitrophenol; 7 = *p*-nitrophenol; 8 = 2,4-dichlorophenol; 9 = 2,4,6-trichlorophenol; 10 = 2,4-dinitrophenol; 11 = 4,6-dinitro-*o*-cresol.

tion of organic mobile-phase component, the other phenol–stationary phase interactions decrease as the acetonitrile fraction increases and begins to competitively solvate solute and stationary phase sites. Separation of a mixture of several phenols is shown in Fig. 2. Reasonably good efficiency and good resolution are observed with the exception of the two components under the third peak.

As the ultimate goal of this work is application to environmental and other practical situations a number of phenols on the EPA Priority Pollutant list were used as samples. Separation of this mixture is shown in Fig. 3. Again, good efficiency and resolution are exhibited.

The QSG-Fe stationary phase has been shown to be useful for the separation of phenolic compounds. The complementary interactions of the solute with the organic backbone and the chelated iron(III) produce good resolution; and, with the application of a gradient for the organic modifier in the mobile phase, phenols with a very wide range of pK_a values can be separated in 15–20 min. Also, the tenacity with which the iron(III) is bound eliminates column bleeding and provides long-term column stability. It is assumed that more effective slurry packing techniques and the use of a silica-gel support with a narrower size range will improve column efficiency. We are attempting to discern, with these factors optimized, whether efficiencies with an iron(III)-loaded QSG column (or any metal, for that matter) will approach those of a good reversed-phase column. Work is continuing to understand more fully the solute (eluent)–stationary phase interactions and to utilize the QSG and QSG-metal columns for other classes of samples.

ACKNOWLEDGEMENTS

We thank the University of North Carolina at Greensboro Research Council, the Research Corporation and the North Carolina Board of Science and Technology for financial assistance with this work.

REFERENCES

1 O. K. Guha and J. Janák, *J. Chromatogr.*, 68 (1972) 325.
2 R. Aigner, H. Spitzy and R. W. Frei, *Anal. Chem.*, 48 (1976) 2.
3 J. J. Latterell and H. F. Walton, *Anal. Chim. Acta*, 32 (1965) 101.
4 D. Kunzru and R. W. Frei, *J. Chromatogr. Sci.*, 12 (1974) 191.
5 R. G. Masters and D. E. Leyden, *Anal. Chim. Acta*, 98 (1978) 9.
6 N. H. C. Cooke, R. L. Viavattene, R. Eksteen, W. S. Wong, G. Davies and B. L. Karger, *J. Chromatogr.*, 149 (1978) 391.
7 F. K. Chow and E. Grushka, *Anal. Chem.*, 50 (1978) 1346.
8 J. R. Jezorek and H. Freiser, *Anal. Chem.*, 51 (1979) 366.
9 C. Fulcher, M. A. Crowell, R. Bayliss, K. B. Holland and J. R. Jezorek, *Anal. Chim. Acta*, 129 (1981) 29.
10 J. R. Jezorek, C. Fulcher, M. A. Crowell, R. Bayliss, B. B. Greenwood and J. Lyon, *Anal. Chim. Acta*, 131 (1981) 223.
11 F. K. Chow and E. Grushka, *Anal. Chem.*, 49 (1977) 1756.
12 J. M. Hill, *J. Chromatogr.*, 76 (1973) 455.
13 H. H. Weetall, *Biochim. Biophys. Acta*, 212 (1970) 1.
14 K. F. Sugawara, H. H. Weetall and G. D. Schucker, *Anal. Chem.*, 46 (1974) 489.
15 G. D. Schucker, K. F. Sugawara and H. H. Weetall, *U.S. Pat.*, 3,886,080 (1975).
16 K. Unger and D. Nyamah, *Chromatographia*, 7 (1974) 63.
17 L. L. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 2nd ed., 1979, p. 206.
18 G. J. Manius and R. T. Tscherne, *Amer. Lab.*, 13 (1981) 138.
19 P. J. Twitchett, A. E. P. Gorvin and A. C. Moffat, *J. Chromatogr.*, 120 (1976) 359.
20 L. G. Sillén and A. E. Martell, *Stability Constants of Metal-Ion Complexes*, Special Publ. No. 25, Chemical Society, London, 1971.