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INVESTIGATIONS INTO THE HPLC SEPARATION OF TETRAALKYLAMMONIUM COBALTO-UNDECATUNGSTOMETALLATES

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ABSTRACT

Mutual separation between $[N(C_7H_{15})_4]_6BCo(H_2O)W_{11}O_{39}$ (AmCo+3B) and $[N(C_7H_{15})_4]_6SiCo(H_2O)W_{11}O_{39}$ (AmCo+2Si) has been achieved for the first time. Reversed-phase C18 liquid chromatography, with 66.7/33.3 MeOH/petroleum ether phosphate buffer was used as the eluent.

Several factors were found to affect the separation. First, the more polar the solvent, the less the retention time will be. Heteropoly compounds can be separated with a certain polarity of the mobile phase. This result appears to be related to the polarity of the solvents. Second, the oxidation number of the cobalt was found to affect the separation. The less the oxidation number of the peripheral hetero atom, the longer the retention will be. The retention time of AmCo+3B was 22.5 min; and the retention time of AmCo+2Si was 31.5 min. As the "pH value" of the phosphate buffer of the 66.7/33.3 MeOH/petroleum ether mixture in the elute increases, the retention times of AmCo+2Si and AmCo+3B decreases. Good chromatographic peaks were obtained when the "pH value" of the buffer was 4.5.

Normal-phase silica liquid chromatography, with 100% methylene chloride, 80/20 CH_2Cl_2/ACN and reversed-phase C18 liquid chromatography with CH_2Cl_2 also were found as possible systems to separate the AmCo+2Si and AmCo+3B samples. But these would require solvent program mode to finish the separation of the samples.

Approved for the Major Division

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I. INTRODUCTION

A. Heteropoly Compounds:

1. Definition of Heteropoly Compounds:

Heteropoly compounds often are known as the compounds containing heteropoly anions. Heteropoly anions contain at least two different kinds of positive valence atoms in addition to oxygen or hydrogen [1]. Of these positive valance atoms, one of them is called the central hetero atom, A. The second atom is called the peripheral heteropoly atom, B. Any other atoms are called the addendum atoms, M. A very general formula of the anions of 12-tungsto heteropolies can be written as:

$$[A_aB_bYM_{(12-b)}O_{(40-b)}]^{q-}$$

where a=1, b=0, 1, 2, 3, 4, 5 or 6; and q=net charge;

Generally, M is tungsten or molybdenum, in its highest oxidation state, +6. The peripheral atom B is cobalt, vanadium, chromium or other first row transitional metal, and Y is the terminal ligand on the hetero atom (Co in this work) [2]. This peripheral atom Co is present in either the +2 or +3 oxidation state. The central hetero atom A may be B, Si, P, Zn, Ga, Ge, or a number of other atoms, including dihydrogen. It is amazing why so many heteropoly anions exist. Sixty-seven different elements [3] can act as either the central or peripheral heteroatoms. These atoms also may be present in different oxidation states within the same reasonably complex heteropoly anion structure.

The hetero atom A can be a non-metal of moderate electronegativity or a metal, especially transition metals, which can exist in a tetrahedral environment. Additional restrictions which appear to be important are that the hetero atom A and addendum atom M must have a favorable combination of ionic radius, charge, and coordination number.

Some differences between the common coordination compounds and heteropoly compounds exist. These include coordination linkage, average charge density, size of addenda atoms, and pi electron acceptor properties of heteropoly compounds. For heteropoly compounds, no discrete ligands are coordinated to the central hetero atom; instead, the structure coordinated to the A atom is interconnected. Also no dissociated fragments of the complex anion coincide with any discrete ligand. In addition, the average density on the surface of heteropoly anions, even with their large overall charge, is very low as compared to that for more traditional coordination compounds. This is due to the large size of the these anions.

2. Historical Background:

The first heteropoly compound was discovered in 1826 when Berzelius noted the yellow precipitate of what we now call 12-molybdophosphate. When he added ammonium molybdate solution to phosphoric acid the compound formed [3]. In 1848, after Svanberg and Struve analyzed the structure of this compound, they postulated that this type of compound was a double salt.

A more precise analytical study of composition of 12-tungstosilicate was done by Marignac in 1862.

The first systematic study of the nature of heteropoly compounds was performed by Miolati in 1908. He suggested a structure of these compounds based on ionic theory and Werner's newly proposed coordination theory. He also developed a theory which was later modified by Rosenheim and became known as the Miolati-Rosenheim (M-R) theory [3].

According to this theory, heteropoly acids are formed around six coordinate hetero atoms with MO_4^{2-} or $M_2O_7^{2-}$ anions as ligands or bridging groups coordinated to this central atom. This theory suggests formulae which are different from those predicted by modern theory. However, these formulae were still used by some people in the 1860's to interpret structures [4].

Early structure characterization of heteropoly compounds came in 1929 when Pauling suggested an alternative to the M-R theory [5]. He proposed a structure for heteropoly compounds having the ratio of the number of central atoms to the addenda atoms as 1:12. According to Pauling's explanation, a stable 12-tungstosilicate heteropoly complex ion can be formed by arranging MO_6 octahedra in such a way that they share corners with each other but not edges or faces. Based on X-ray powder crystallographic studies, Keggin derived more detail of the anion structure. In 1933, he suggested that octahedral units were joined together by shared edges and corners [6]. This structure is essentially the correct structure as we know it today. The structure, known today as the "Keggin structure", is shown in Figure 1_a utilizing polyhedra. The corners of twelve octahedral and the one tetrahedron represent the oxygen atoms. The tungsten atoms and other heteroatoms are located at the centers of the polyhedra. This structure has tetrahedral symmetry when b of the general formula is zero. Because the Keggin structure is basis of a large classes of heteropolies, the Keggin structure was later verified, in 1936 by Bradley and Illingworth, using single crystal X-ray methods.

Almost twelve years passed before, in 1948, Evans reported the next crystal structure of a different kind of heteropoly, the $[TeMo_6O_{24}]^{6-}$ anion [7]. Soon a number of other different structures were known.

In 1962, a tungsten heteropoly anion was made by Simmons which contained two different heteroatoms, cobalt and silicon. She confirmed that tungsten atoms were the addenda atoms and that both Co and Si atoms were part of the anion and not just counterions [8]. But it was impossible to determine whether this anion contained one cobalt per 11 tungsten atoms or one cobalt per 12 tungsten atoms due to analytical difficulties. Baker, Simmons, and their colleagues suggested that this anion was derived from the Keggin structure and that the ratio of the formulation of these anions was 1:1:11 [9].

In 1970, Figgis [10] proposed that the 1:1:11 of Simmons should be formulated as $K_6[SiCoH_2OW_{11}O_{39}]$ including two non-acidic hydrogen atoms as part of the anions. These two hydrogen atoms were proposed to be part of a water ligand connected to cobalt in the anion. The structure of this anion can be represented as in Figure 1_b. In this formulation, the cobalt is in pseudooctahedral coordination. The cobalt with an attached water ligand was substituted for a tungsten addenda atom and its external oxygen. The symmetry is reduced from T_d and C_s.

In 1977, Landis [11] further substantiated this interpretation during his study of the octahedral trans effect of cobalt using a series of these 1:1:11 diheteropoly tungstate salts. He confirmed the 1:1:11 formula for the dihetero-11-tungsto anions and further substantiated that the water molecule was coordinated to the cobalt atom by preparation, analysis, and characterization of thirty-two potassium salts of dihetero-11-tungsto anions. A typical formula of one of these dihetero-11-tungsto anions is $[GeCo(N_2C_4H_4)W_{11}O_{39}]^{5-}$, where the pyrazine ligand, $N_2C_4H_4$, has replaced the water ligand. This anion can be named as pyrazinecobalto(III)-11-tungstogermanate. Landis in his work, in order to emphasize the pseudooctahedral coordination of cobalt and the tetrahedral coordination of the central heteroatom wrote these formulae as $[N_2C_4H_4CoO_5W_{11}O_{30}GeO_4]^{5-}$.

In this work, the water ligand of analyzed salts (analysis of all elements except oxygen were performed) was substituted by various amine ligands. The product compounds also were analyzed for all elements except oxygen. The $[AW_{11}O_{39}]^{q-}$ part of the anion can be treated as a pentadentate ligand because this anion with a hole can be isolated. Because of this hole, workers in this field use the term lacunary to describe this type of species. His investigations of the octahedral trans effect of cobalt complexes using these lacunary heteropoly tungstate anion ligands also showed that the central A heteroatom has effects on the amines coordinated trans to this heteroatom. He determined this by studying the measurements of the UV, visible, near IR, IR, and proton NMR spectra of a number of analogous 1:1:11 anions.

In 1989, the solubilities in organic solvents of five diheteropoly tungstates of the Keggin structure were investigated by Zaidi [12]. In his thesis, he reported that potassium salts of water soluble anions -- aquocobalto(II)-11-tungstosilicate, aquocobalto(II)-11-tungstogermanate, aquocobalto(III)-11-tungstoborate, aquocobalto(III)-11-tungstogallate, and aquocobalto(III)-11-tungstozincate -- were converted to salts which were soluble in organic solvents. This was accomplished by mixing the potassium salts with tetra-n-heptylammonium bromide in a cyclohexane-water mixture.

In his work, the absorption spectra of these five compounds also were determined in eight selected organic solvents -- cyclohexane, toluene, carbon tetrachloride, chloroform, methylene chloride, isopropyl alcohol, tetrahydrofuran, and acetonitrile. Most of the UV spectra of tetra-n-heptylammonium heteropoly compounds in solution exhibited two intense peaks. Most of these were in the range 250 - 280 nm. The visible spectra exhibited multiple peaks, indicative of pseudooctahedral cobalt(II) or cobalt(III) chromophores, and the wavelength of maximum absorbance was noted to shift with change of solvent polarity.

3. General Properties of Heteropoly Compounds:

In this section, some general properties of heteropoly compounds are given, more details of the properties of 1:12 heteropoly compounds of tungsten are given in papers by Baker, Pope, and others [1-4, 13, 14].

a. Heteropoly tungstates are more stable in acidic than neutral solutions. All heteropoly compounds are decomposed in concentrated base solution.

b. Heteropoly tungstates are unstable when the temperature is higher than 90°C, and will lose water molecules when the temperature is higher than 150°C. Complete decomposition occurs at about 500°C.

c. The stability of heteropoly compounds, based on the size of the hetero atom A and the charge on the heteropoly anions, increases as the charge on heteropoly anions decreases. Heteropoly tungstates compounds are hydrolytically more stable than the corresponding molybdenum compounds.

d. Salt formation between heteropoly anions and polycations is not just ionic bonding. Although the electrostatic attractions due to the high negative charge of the heteropoly anions generally associated with the polycations exists, strong ion pairing is expected in solution.

e. The color of heteropoly compounds is based on the peripheral hetero atom, B, and/or addendum atom, M. In general, 1:12 heteropoly compounds of tungsten are colorless.

f. Heteropoly acids show very strong affinity toward other oxygen donor ligands.

g. The measurements of ionic weight by light scattering in aqueous and organic solvents and of ionic size by ultracentrifugation, viscosity, and density indicate that 1:12 heteropoly acids are monomeric in nature.

h. Typically, polyoxoanions are air stable species of large size (0.60 - 2.50 nm) and high ionic weight (1,000 - 10,000). For $[SiW_{12}O_{40}]^{4-}$, the ionic weight is 2876.

i. The free acids and many of their salts are readily dissolved in water. Aqueous solutions of these salts are very dense due to their high solubilities and high molecular weights. Some metal salts are relatively insoluble. Usually the larger the size of cation, the more soluble is its salt with a given heteropoly anion. Low lattice energy of heteropoly compounds and low energy of solvation of anions cause the heteropoly compounds to be more soluble in water.

j. The free 1:12 heteropoly acids do not dissolve in non-oxygenated solvents such as benzene, chloroform etc.

k. Crystalline heteropoly acids are highly hydrated, they can combine with up to 50 molecules of water per anion. Much of this water is zeolytic in nature. Also, the hydrated acids usually have isomeric structures.

1. Some heteropoly compounds are strong oxidizing agents which are easily reduced to stable compounds. The color of these reduced compounds is very intense. These compounds of mixed valance are known as heteropoly blues. The reduced compounds can in turn act as reducing agents. The original color of the compound is kept on oxidation.

4. Some Important Applications of Heteropoly Compounds:

Applications of heteropoly compounds are based principally on their catalytic properties. Thermal stabilities, redox properties, solubilities, and solvolytic behavior in both aqueous and organic media are used in various other applications [3, 4].

a. Catalysis:

Heteropoly compounds act as heterogeneous catalysts in a number of reactions. For example, 12-tungstophosphoric acid and 12-tungstosilicic acid are reported as the most effective catalysts for dehydration of castor oil to unsaturated oils. Furthermore, 12-tungstophosphoric was utilized as the catalyst in the hydroxylation of alkyl alcohol into glycerol. Catalytic oxidation of lower olefins into unsaturated aldehydes using various heteropoly compounds as the catalyst have been studied [3]. Heteropoly compounds have been utilized as catalysts for the vapor-phase partial oxidation of naphthalene and the vapor-phase hydration of ethylene. Solid heteropoly acids have been reported as the efficient catalysts for polymerization olefins and benzyl alcohol.

Much work on catalytic processes involving heteropoly anions in homogeneous solution also have been done. This is also a broad field to study. Because of the difficulty in ascertaining the species actually involved in the catalyst, less papers appear to have been published.

b. Chemical Analysis:

Heteropoly anions are useful in gravimetric and colorimetric analytical methods for phosphate, silicate, arsenate, and germanate ions. These methods rely on the reduction and precipitation properties of heteropolies [15]. These ions can be analyzed separately or together by forming the corresponding heteropoly anion first, then selectively extracting anion using organic solvents at appropriate pH values.

c. Ion-Exchangers:

Crystalline heteropoly compounds can be used as ion-exchangers in thin-layer chromatography because of their porous crystalline structures. The counter ions readily move through the structure. The ion-exchange occurs throughout the crystal lattice and not just on the surface of crystals.

d. Biochemical:

Cholesterol and uric acid can be determined with heteropoly anions using colorimetric analytical methods.

Phosphotungstic acids are utilized as the analytical colorimetric reagent of choice for proteins, alkaloids, and purines. Alternatively these acids can be used as precipitants for proteins. The acids can be used as a non-specific dense strain for electron microscopy [16]. The latter two applications depend on columbic interactions between the polyanion and cationic sites on the biomolecules.

e. Corrosion Inhibition:

Heteropoly compounds are good intermediate coatings for steel and aluminum. As inorganic coatings on steel, they have anticorrosion properties. Some heteropoly compounds undergo hydrolytic degradation in very dilute solutions so that this kind of coating must be used for special situations.

f. Flame Retardant:

Molybdenum compounds are useful as flame retardants for wood and textiles. Also, these compounds can be used as smoke suppressants in textiles and plastics.

g. Other Applications:

Heteropoly acids are good protonic conductors and are electrochromic in the solid state. Hence heteropoly blues can be made. These heteropoly blues can be used as the identifying reagent for several organic radicals generated upon radiolysis of aqueous solutions.

B. High Pressure Liquid Chromatography (HPLC):

Only about 20% of the known compounds lend themselves to analysis by gas chromatography either because they are insufficiently volatile and cannot pass through the column or because they are thermally unstable and decompose under the conditions of separation [17, 18]. High-performance liquid chromatography (HPLC) is not limited by sample volatility or thermal stability. The HPLC technique is able to separate macromolecules, ionic species, labile natural products, polymeric materials, and a wide variety of other high-molecular-weight, polyfunctional molecules and ions. In comparison to gas chromatography, HPLC has an interactive liquid mobile phase that is not available in gas chromatography as an additional parameter to an active stationary phase for selectively separating the species. Chromatographic separation in HPLC is the result of specific interactions that are essentially absent in the mobile phase of gas chromatography. A greater variety of stationary phases, which allows a greater variety of these selective interactions, affords more possibilities for separation with HPLC.

The HPLC technique involves several factors which are very important in affecting the degree to which two or more substances can be separated. Four of the most important of these are the number of theoretical plates (N), the capacity factor (k'), the selectivity factor (alpha), and the resolution (R).

The number of theoretical plates (N) is a measure relating chromatography to organic chemistry. One theoretical plate is analogous to the separation power of one simple distillation. Therefore, N is a representation of how many simple partitions occur as the solute passes through the column. The value of N can be calculated from the following equation:

$$N=5.54*(T'_r/W_{1/2})^2 \qquad \text{(eq. 1)}$$

where T'_r equals the adjusted retention time and $W_{1/2}$ is equal to the peak width at half height.

The capacity factor (k') is an expression of how many void volumes (the space between the particles) are required for a given compound to elute. The time required to elute the desired solute (T'_r) divided by the time required for a non-retained solute to elute (T_o). The ratio of the capacity factors for two compounds is called the selectivity factor (alpha). These two factors are represented by the following:

$$k'=(T'_r/T_o) \qquad \text{(eq. 2)}$$

$$alpha=(k'_2/k'_1) \qquad \text{(eq. 3)}$$

The resolution (R) of two compounds is a measure of the degree to which separation of peaks can be achieved. Values of R are a good indicator of how feasible a separation will be. A value of approximately one or higher is theoretically needed for peak separation to occur. The mathematical expression for R is :

$$R=(T'_{r2}-T'_{r1})/[0.5*(W_2+W_1)] \qquad \text{(eq. 4)}$$

where W_2 and W_1 are the base widths of the two compounds.

The detection limit (C_d) of a compound can also be determined using HPLC. This can be done by measuring peak heights X_i for various concentrations of analyte and comparing them with the standard deviation of the baseline noise. The detection limit is defined as:

$$X_d=X_b+3*S_b \qquad \text{(eq. 5)}$$

where X_d is the detection limit of the response, X_b is the signal of the blank, and S_b is the standard deviation of the blank signal. The concentration C_d corresponding to the response is the detection limit.

C. Goals of this Project:

As was noted earlier, some 1:1:11 heteropoly salts have been converted to salts which are soluble in organic solvents. This discovery increases the number of reactions in which heteropoly compounds can be investigated. One area is chromatography, especially HPLC. In HPLC the subtle interactions which occur as the liquid mobile phase is changed are very important. These subtle interactions should allow for the selective separation of 1:1:11 heteropoly anions, ions which differ only in small ways. For example, changing the interior heteroatom A from Si to Ge, the overall anion charge does not change; and the valence electrons do not change. Even though the size of the A atom does change, the overall anion size remains essentially the same.

1. The Primary Goal of This Project:

The usual HPLC adsorbents used are C18 adsorbent and silica adsorbent. These adsorbents were available in our lab and usually are available in other labs. Hence these were to be used first rather than less common adsorbents. If C18 or silica can be used, more researchers will have access to the proposed analytical technique.

In order to have a good chance of succeeding yet still not have compounds which are too dissimilar, two compounds were chosen such that the overall charge was same (-6), yet the peripheral hetero atom B was in different oxidation states. These conditions require that the central hetero atom A either have different oxidation states or be from different families of the periodic chart. The latter was chosen. The compounds used were the tetra-n-heptylammonium salts of the anions $[BCo(H_2O)W_{11}O_{39}]^{6-}$ and $[SiCo(H_2O)W_{11}O_{39}]^{6-}$.

2. The Second Goal of This Project:

The differences between the two selected heteropolies is small and even more subtle differences exist between other heteropoly anions, it was hoped that a more selective adsorbent than C18 or silica could be found. A survey of packing materials commercially available was to be conducted.

3. The Third Goal of This Project:

Find a better separation by using the various solid phase cartridge systems (SPCS) and solvents.

4. The Fourth Goal of This Project:

Finding the best composition of solvent mixture of those tested to separate heteropoly compounds in HPLC after the previous goals were accomplished.

II. EXPERIMENTAL

A. Apparatus:

1. High Pressure Liquid Chromatograph (HPLC):

All data were taken on a Varian high pressure liquid chromatograph, Varian model 2010 pump equipped with a Varian model 2050 detector. A reverse phase C18 column or a silica column (25cm X 4.6mm i.d.) was used to affect the separation. All outputs were recorded on Alltech model 261 with 10mV full scale stripchart recorder.

2. Refractometer:

A Bauch & Lomb refractometer was used to measure the refractive indexes of mixed composition solvents.

3. Balance:

A Gram-atic balance (E. Mettler Switzerland) accurate to 5 decimal places with a sensitivity of 0.01 mg was used.

4. pH meter:

A digital pH pen was used for measuring "pH values" of solutions. Since the solutions were not aqueous, the actual hydrogen ion activity (i.e. the pH) are not known. In this thesis quotation marks will be used around pH values to emphasize this point.

5. Solid Phase Cartridge System (SPCS):

Solid Phase Cartridge System (SPCS) adsorbents purchased from Alltech Associates, Inc. were utilized for protecting HPLC instrument. These consisted of 100 mg of various solid phases in plastic tubes. These SPCS adsorbents also were used for preliminary screening tests.

6. Other Glass Apparatus:

A 10 μl HPLC syringe (Hamilton Co.) was used to load the HPLC injector. Various volumetric and graduated pipets, (e.g., 1.0 ml, 5.0 ml, 10 ml, and 25 ml) from Kimax were used for solution preparations. A vacuum filter flask was used to increase the pressure differential of the SPCS system and increase the flow rate. Small test tubes and vials were used in the preparation of some solutions.

7. Other Apparatus:

A stainless steel micro spatula was used for transferring and handling the solid chemicals. Special care was used to avoid moisture when transferring the heteropoly compounds so that the metal micro spatula would not react with the tungsten heteropoly compounds.

B. Chemicals:

1. Nomenclature:

The names of organic solvents used in the previous sections were those used by the suppliers. In this thesis, these names will be retained rather than using the IUPAC names [19].

Abbreviations will be used in this thesis due to the length and complexity of the heteropoly anion formulae and names. For example, potassium aquocobalto(II)-11-tungstosilicate, $K_6[H_2OCoO_5W_{11}O_{30}SiO_4]\bullet 15.2H_2O$, will be abbreviated as KCo+2Si and the anion of this salt will be abbreviated as just Co+2Si. The second to last symbol, Co+2 will represent the atom cobalt and its oxidation state, +2. The last symbol Si will represent the central tetrahedral atom. Similarly, Co+3B implies the anion, $[H_2OCoO_5W_{11}O_{30}BO_4]^{6-}$; and AmCo+3B implies the tetra-n-hetpylammonium heteropoly compound of this anion.

2. Heteropoly Salts:

Hydrated potassium salts were provided by Dr. Art Landis. These salts had been analyzed for volatiles (water) and all elements expected in the particular salt except oxygen. Hence exact amounts of compounds could be measured by mass. The formulae of these salts and the abbreviations used in this thesis are shown below:

a. Potassium aquocobalto(II)-11-tungstosilicate

Formula: $K_6[H_2OCoO_5W_{11}O_{30}SiO_4]\bullet 15.2H_2O$

Notation: KCo+2Si

b. Potassium aquocobalto (III)-11-tungstoborate

Formula: $K_6[H_2OCoO_5W_{11}O_{30}BO_4]\bullet 16.8H_2O$

Notation: KCo+3B

3. Organic Ammonium Salts:

Tetra-n-heptylammonium bromide (Eastman Kodak Co.) was used without further purification.

4. Solvents:

Distilled, deionized water was used as the aqueous solvent. Mixtures of phosphic acid and sodium dihydrogen phosphate were used as buffers.

Other solvents used, the abbreviation used in this thesis, and their sources are listed below:

Acetonitrile (ACN) (Fisher Scientific Co.)

Carbontetrachloride (CCl_4) (Fisher Scientific Co.)

Cyclohexane (C_6H_{12}) (Taylor Chemical Co.)

Ethyl acetate (EtOAc) (Aldrich Chemical Company, Inc.)

Methanol (MeOH) (Fisher Scientific Co.)

Methylene chloride (CH_2Cl_2) (Fisher Scientific Co.)

Petroleum ether (Petro. ether) (Fisher Scientific Co.)

Toluene ($PhCH_3$) (Fisher Scientific Co.)

All chemicals were ACS reagent grade or HPLC spectral grade unless otherwise noted. All samples and solvents were filtered through 0.22 μm filter paper prior to use in the HPLC instrument.

C. Preparation of Solutions:

1. Preparation of Ammonium Salts:

The potassium heteropoly salts used in this research were converted into tetra-n-heptylammonium salts utilizing a technique which we call liquid ion exchange phase transfer [12]. The following are the procedures:

A sample of 1.0 g tetra-heptylammonium bromide was placed in a 25 ml test tube along with 1.0 g of the heteropoly to be extracted.

Approximately equal volumes (1-2ml) of methylene chloride and water were placed in the test tube and the resulting three phase mixture stirred for 1-2 hours.

At first, a colored solution was present in the bottom water layer. Upon stirring, the color gradually moved to organic top layer. This organic layer was then drawn off and the solvent is evaporated using a sand bath at 50°C. A colored solid was obtained after evaporating all organic solvent. Because tests and experience indicated that methylene chloride was a good solvent for these new tetra-n-heptylammonium heteropoly compounds [12], all these compounds were twice redissolved and reevaporated from methylene chloride prior to use. This procedure also assured that the starting organic heteropoly had the same composition.

2. Preparation of Mobile Phases:

All mobile phases were prepared by volume/volume ratios (before mixing). The mixed solutions were filtered through 0.22 μm filters prior to use.

D. Solid Phase Cartridge System and HPLC Procedures and Experimental Techniques:

All experiments used tetra-n-heptylammonium aquocobalto(II)-11-tungstosilicate (AmCo+2Si) and tetra-n-heptylammonium aquocobalto(III)-11-tungstoborate (AmCo+3B) in methylene chloride solution as the samples.

1. Solid Phase Cartridge System (SPCS):

Screening tests were performed by first passing two or three bed volumes (0.60 ml) of the desired solvent through the column. Next the sample was put onto SPCS, followed by two or three bed volumes of solvent to elute the sample. The eluted solvent was evaporated from the eluted sample using a sand bath. The recovered solid was then reused (because the samples were limited and expensive).

This method was used with each of the following solvents on the SPCS -- CCl_4; CH_2Cl_2; MeOH; ACN; toluene; cyclohexane; 90/10 and 80/20 EtOAc/petroleum ether; 90/10, 80/20 and 50/50 EtOAc/cyclohexane; 90/10 and 80/20 CH_2Cl_2/MeOH; 50/50 EtOAc/CH_2Cl_2; 90/10 and 80/20 CH_2Cl_2/ACN; and 80/20, 50/50 MeOH/petroleum ether. The packing materials in the SPCS were C18, Silica, Diol, Amino, SAX (Anion exchange) and SCX (Cation exchange) solid phases. Not all possible combination were tested.

2. HPLC [20, 21]:

All injections were made utilizing a 10 microliter feedloop. All flow rates of mobile phase were 1.0 ml/min except as noted elsewhere in this thesis, the chart speed was 1.0 cm/min, and the detector was set at 270 nm. Hence on the chromatograms each centimeter represents 1.0 ml. All injections were made at least twice.

a. C18 Column:

The injections of the heteropoly samples were done using the following mobile phases -- 90/10, 80/20, and 50/50 EtOAc/Cyclohexane; 90/10, 80/20 and 92/8 CH_2Cl_2/ACN; 50/50, 66.7/33.3 MeOH/petroleum ether, 66.7/33.3 MeOH/petroleum ether with various "pH values" as measured in the filtered solution. The "pH values" used were "pH"=7.3, and "pH"=4.5. Since petroleum ether is a mixture whose composition can vary attempts were made to find a more reproducible substitute. The solvents -- hexane; octane and hexane; hexane and pentene; and pentene, hexane, and octane mixtures -- were made to find a substitute for the petroleum ether. These mixtures were mixed with MeOH according to the above-mentioned ratio as the mobile phase. The MeOH/petroleum ether mixture was a true solution. However, some of the above mixtures with MeOH are not miscible.

b. Silica column:

The injections of the heteropoly samples were done using the following mobile phases -- ACN; ACN and two drops of EtOAc in samples; CH_2Cl_2; and 95/5, 90/10, 50/50, 45/55, 35/65, 32/68, 27/73 and 23/77 CH_2Cl_2/ACN as the mobile phases.

III. RESULTS

The color of AmCo+2Si is red, and the color of AmCo+3B is bright green. These colors were used to help identify the compounds.

A. Solid Phase Cartridge System (SPCS):

In this section, The SPCS system were used to rapidly test the separation of AmCo+2Si and AmCo+3B from a number of adsorbents. The packing materials C18, Silica, Diol, NH_2 (Amion), SAX (Anion Exchange), and SCX (Cation Exchange) are described below in order. In each case the results with each mobile phase solvent will be described in the order of approximately increasing polarity. The order is cyclohexane, carbon tetrachloride, toluene, methylene chloride, ethyl acetate, methanol, and acetonitrile.

1. C18 Column:

Neither AmCo+2Si nor AmCo+3B samples eluted from C18 column after ten bed volumes of cycloheaxane were added.

With toluene mobile phase neither AmCo+2Si nor AmCo+3B samples eluted from C18 column after using ten bed volumes.

Although AmCo+2Si and AmCo+3B can be dissolved in methylene chloride quite well, neither AmCo+2Si nor AmCo+3B eluted from the C18 column after ten bed volumes of methylene chloride were used.

Three bed volumes of the mixture of 90% ethyl acetate and 10% petroleum ether eluted both the AmCo+2Si and the AmCo+3B samples from the C18 column. The color of samples didn't change after the solvents were evaporated at 90°C (the sample was decomposed over 90°C).

However ten bed volumes of the mixture of 80% ethyl acetate and 20% petroleum ether eluted only part of samples.

Both AmCo+2Si and Amco+3B eluted from the C18 column using three bed volumes of various mixtures of ethyl acetate and cyclohexane. The ratios of mixtures were 90/10, 80/20, and 50/50.

Both AmCo+2Si and AmCo+3B samples were eluted from the C18 column after three bed volumes of methanol were used.

Three bed volumes of both 90/10 and 80/20 mixtures of methylene chloride and methanol, used separately, eluted AmCo+2Si and AmCo+3B from the C18 column.

Three bed volumes of acetonitrile, used as the mobile phase, quickly eluted both the AmCo+2Si and the AmCo+3B samples.

Both AmCo+2Si and AmCo+3B eluted from the C18 column after three bed volumes of 50/50, 80/20, and 90/10 mixtures of methylene chloride and acetonitrile were used as the mobile phase.

The AmCo+2Si sample eluted from the C18 column after three bed volumes of a 66.7/33.3 mixture of methanol and petroleum ether were used. The AmCo+3B sample also eluted from the C18 column after only two bed volumes of the above solvent were added. In addition, three bed volumes of a 50/50 mixture of methanol and petroleum ether were used. Both the AmCo+2Si and the AmCo+3B samples eluted from C18 column.

These C18 results are summarized in Table I.

2. Silica Column:

When ten bed volumes of the cyclohexane were used as the mobile phase with the silica column, neither AmCo+2Si nor AmCo+3B eluted.

With methylene chloride mobile phase, AmCo+2Si eluted from the silica column after three bed volumes of methylene chloride were used. However AmCo+3B did not elute from the silica column even after ten bed volumes of methylene chloride were added.

Both AmCo+2Si and AmCo+3B rapidly eluted from silica column after three bed volumes of acetonitrile were used as the mobile phase.

Both AmCo+2Si and AmCo+3B eluted from silica column after three bed volumes of 90/10 and 50/50 mixtures of ethyl acetate and cyclohexane were used.

When three bed volumes of 90/10, 80/20, 50/50, and 10/90 mixtures of acetonitrile and methylene chloride were used as the mobile phase, both AmCo+2Si and AmCo+3B eluted from silica column. On the other hand, when 5/95 and 2/98 mixtures of acetonitrile and methylene chloride were used, the AmCo+2Si sample eluted from silica column after three bed volumes. The AmCo+3B sample did not elute from silica column, even though ten bed volumes of mixture solvents were used.

Both AmCo+2Si and AmCo+3B quickly eluted from the silica column after three bed volumes a 50/50 mixture of acetonitrile and water were used as the mobile phase. But some turbidity appeared in the eluted AmCo+3B solution.

These silica results are summarized in Table II along with data for other adsorbents.

3. Diol column:

Both AmCo+2Si and AmCo+3B were eluted from the diol column by using three bed volumes or less of cyclohexane; a mixture of 80/20 methylene chloride and cyclohexane; methylene chloride, or a mixture of 80/20 methylene chloride and acetonitrile respectively. These diol results are also summarized in Table II.

4. NH_2 (Amino) Column:

Three bed volumes of a mixture of 80/20 methylene chloride and cyclohexane completely eluted both AmCo+2Si and AmCo+3B from the NH_2 column.

5. SAX (Anion Exchange) and SCX (Cation Exchange) Columns:

Both AmCo+2Si and AmCo+3B completely eluted from the SAX column and SCX column after three bed volumes of methylene chloride were used as the mobile phase.

The results obtained with the SPCS for these latter three packings along with that data are included in Table II.

B. High Pressure Liquid Chromatography (HPLC):

In this section, the chromatographic results from separate injections of the AmCo+2Si and AmCo+3B samples into the various mobile phases using either of the two columns, C18 and

Silica will be described. Because the polarity of solvents are quite different, the chromatographic results sometimes exhibit four peaks, sometimes three peaks, and sometimes two peaks, and sometimes even one peak or no peak.

1. Silica Column:

a. Acetonitrile:

Three peaks appeared on the chromatogram when the AmCo+2Si was injected, the first peak was very strong, the second and third peaks are weak and close (almost overlap). With the injection of AmCo+3B sample, four peaks appeared on the chromatogram. The first two peaks are very strong; and the latter two peaks were relatively weak. These four peaks were not separated based at the baseline although they were so close to baseline separation. As can be seen in Figure 2 and Table III, the retention time of first three peaks of the two samples are almost same. The similar results were obtained (see Figures 3 and 4) when the acetonitrile was used as mobile phase with the same conditions. However three peaks appeared on the chromatogram when the AmCo+2Si sample, including two drops of ethyl acetate, were injected; and two peaks appeared on the chromatogram with the injection of the AmCo+3B sample. The retention time of both samples are almost same (see Table III). One peak was less intense than the added ethyl acetate to AmCo+2Si and AmCo+3B solutes (see Figure 5).

b. Methylene Chloride:

One peak appeared on the chromatogram of the injection of peaks were weak and close. These three peaks were separated completely at the baseline (see Figure 10).

In the 45/55 and 35/65 CH_2Cl_2/ACN mixtures, almost the same results were obtained with both mobile phases. Two peaks appeared on the chromatograms for the injection of the AmCo+3B and the AmCo+2Si solutes. The first peak is very weak and only one other peak appeared in the chromatograms. The retention times of first peak in the two samples were same (see Figure 11 and 12 and Table III).

Two peaks appeared on the chromatograms of the 27/73 and the 23/77 CH_2Cl_2/ACN mobile phases when the AmCo+3B and AmCo+2Si samples were injected. The retention times of AmCo+2Si and AmCo+3B were different, but they were close. The results obtained from two trials can be seen in Figures 14 and 15 and in Table III.

The values of retention times obtained from all the chromatographic results utilizing the silica column can be seen in Table III. The composition of mixtures of methylene chloride and acetonitrile were determined using a set of standard mixtures. The refractive indexes of the standard mixtures and the experimental mixtures are given in Table IV. The calculated concentrations are also given in the Table IV.

2. C18 Column:

a. Mixtures of 50/50, 80/20, and 90/10 Ethyl Acetate and Cyclohexane:

Two peaks appeared on the chromatograms when AmCo+3B and

AmCo+2Si were separately injected using the 50/50 EtOAc/C_6H_6 mixture as the mobile phase. As can be seen in Figure 16, the second peak was weaker than the first peak, and the retention times of the second peak of both samples were same. Almost similar results were obtained by utilizing the mixture of 80/20 ethyl acetate and cyclohexane (see Figure 17).

Three peaks appeared on the chromatograms when AmCo+2Si and AmCo+3B solutes were injected separately in 90/10 ethyl acetate and cyclohexane mobile phase. Compared to the first two peaks, the third peak of both samples was quite weak. The retention times of all three peaks of both solutes were almost same (see Figure 18 and Table V).

b. Mixtures of 80/20, 90/10, and 92/8 methylene chloride and acetonitrile:

When 80/20 methylene chloride and acetonitrile mixture was used as the mobile phase, one peak appeared on the chromatograms when AmCo+2Si solute was injected, and two peaks appeared on the chromatograms when AmCo+3B solute was injected. The retention times of the single peak of the AmCo+2Si solute and of the second peak of AmCo+3B solute were almost same. These results are showed in Figure 19 and Table V.

Similar results were obtained with the injection of AmCo+3B and AmCo+2Si using the mixture of 90/10 methylene chloride and acetonitrile as the mobile phase. The retention times of the single peak of the AmCo+2Si solute and of the second peak of the AmCo+3B solute were almost same (see Figure 20).

Two peaks appeared on the chromatogram using 92/8 CH_2Cl_2/ACN mixture as the mobile phase when AmCo+3B solute was injected, but no peaks appeared on the chromatograms when AmCo+2Si solute was injected. The chromatographic results can be seen in Figure 21.

c. Mixture of 50/50 and 66.7/33.3 Methanol and Petroleum Ether:

One peak appeared on the chromatogram when AmCo+3B solute was injected using 50/50 MeOH/petroleum ether was used as the mobile phase. Three peaks appeared on the chromatogram for the AmCo+2Si solute. The first two peaks of AmCo+2Si solute were very weak. The second peak and the third peak were not separated completely; they were joined together (see Figure 22). The retention times of the AmCo+3B peak and the second peak of the AmCo+2Si solute were almost same (see Figure 22 and Table V).

Using the 66.7/33.3 MeOH/petroleum ether mixture as the mobile phase, two peaks appeared on the chromatograms when AmCo+2Si and AmCo+3B solutes were injected respectively. As can be seen in Figure 23 and Table V, the retention times of second peak of both samples were quite different. The retention time, expressed in milliliters, of the AmCo+3B solute (2.4 mL) was less than the retention time of the AmCo+2Si (3.2 mL).

When a mixture of AmCo+2Si and AmCo+3B solutes was injected using the 66.7/33.3 methanol and petroleum ether as the mobile phase, three peaks appeared on the chromatogram. The first peak was very weak. The second and third peaks were reasonably well separated but not at the baseline. These two peaks exhibited had some tailing and were not smooth. The "pH value" of the 66.7/33.3 methanol and petroleum ether mixture was 7.3. When the "pH value" of the 66.7/33.3 MeOH/petroleum ether mixture was adjusted to 4.5, the chromatograms of the individual solutes exhibited no tailing and the peaks were sharp and smooth (see Figure 25). The retention times of both samples, still are quite different (see Figure 25). The values, expressed in milliliters, are 2.6 ml and 3.1 ml respectively. Using this mobile phase three peaks appeared on the chromatograms when the mixture of AmCo+3B and AmCo+2Si solutes was injected. The retention volumes of these three were quite different (1.6, 2.6, and 3.1 ml). These results were obtained from several trials (see Figure 26 and Table III).

The values of retention times obtained from all the chromatographic results using C18 are given in Table V. Values of six chromatographic parameters ($W_{1/2}$, W_b, N, k', alpha, and R) are given in Table VI for the 66.7/33.3 MeOH/petroleum ether mobile phases. The resolution factor for the two solutes is 0.8 in the "pH" 7.3 mobile phase and 1.5 in the "pH" 4.5 mobile phase.

IV. DISCUSSION

Analysis of the data in Tables III and V obtained from all the experiments yielded several important results. Most importantly, the 66.7/33.3 MeOH/petroleum ether mixture at "pH" 4.5 is the best mobile phase of those tested for the separation of heteropoly compounds using C18 column. These results are consistent with the results obtained from SPCS system with the C18 packing (see Table I). To achieve this separation, the AmCo+3B and AmCo+2Si solutes first were eluted separately using 66.7/33.3 MeOH/petroleum ether in a SPCS C18 system packing. Different volumes were required to elute the samples. This indicated that these samples probably could be separated using the HPLC instrument. Next, chromatographic results of separate injections of AmCo+3B and of AmCo+2Si solutes showed that the retention times of the AmCo+3B and AmCo+2Si solutes in this mobile phase were quite different. Thus when the mixture of AmCo+3B and AmCo+2Si was injected, two peaks appeared and were almost separated on the first attempt. This meant that the AmCo+2Si and AmCo+3B almost could be separated by 66.7/33.3 methanol and petroleum ether mixture in C18 column on HPLC. But some problems still existed, the peaks of AmCo+3B and AmCo+2Si solutes obtained using this mobile phase were tailing and not smooth.

Some references [22, 23, 24] were found which reported that the "pH value" of mobile phase would affect the separation of 1:12 heteropoly compounds. Therefore some adjustment (using sodium dihydrogen phosphate and phosphoric acid buffer) of "pH values" of the mobile phase were tried in attempts to improve the separation of AmCo+3B and AmCo+2Si solutes. The shape of the peaks were improved dramatically (see Figure 26) when the "pH value" of the mobile phase was changed to 4.5 units. These results further proved that the "pH value" of the mobile phase would affect the separation of the heteropoly compounds.

When comparing the corresponding chromatographic parameters of Table VI, all values for these factors were higher for 66.7/33.3 MeOH/petroleum ether at "pH" 4.5 than at "pH" 7.3 for this mobile phase. One more thing needs to be noted, petroleum ether is a complex mixture which is composed of several organic solvents including hexanes, heptanes, pentanes, hexenes, heptenes, pentenes and so on. Because the compositions of the petroleum ether solvents may vary from batch to batch or bottle to bottle, the reproducibility of separation in future studies probably will be slightly different. Therefore some kind of combination of the above mentioned organic solvents would be better than the petroleum ether for solvent for mixing with methanol to prepare the mobile phase for separating the heteropoly compounds. Oddly petroleum ether and methanol are miscible at the ratios used. However most of the above components of petroleum ether are not miscible with methanol. The miscibility of petroleum ether was assumed to be brought about by the more polar components such as alkenes. Attempts to find a more reproducible mobile phase were unsuccessful because of the limited number of organic solvents available during this phase of the research (only pentene, no hexene and heptene). Further study should be focused on this problem. Chromatograms of AmCo+3B and AmCo+2Si samples with this eluent, are reproducible and exhibit baseline separation (Figure 26). The retention times for duplicate runs differ by 0.05 min or less (refer to Table VI). These differences (approximately 3 seconds) are too large to have been caused by differences in injection techniques or by fluctuations in flow rate. However a combination of these factors and others, such as line thickness on the chart paper, could account for this error. The errors in the peak widths (0.02 min) is within our ability to reproduce the measurements on the same chromatogram, let alone on the duplicate chromatograms.

The capacity factors (k') are slightly less than one would expect for good separation. Perhaps the void volumes (or retention time for an unretained species T_0) is too large. The value of 1.60 mL was taken as the first deviation from baseline after injection. This peak should be the solvent used for the solutes. Presumably the solvent is not retained. The 1.60 ml void volume appears to be a reasonable value based on other kinds of chromatography with a C18 column of this type.

During the research some additional good solvents also were found as mobile phases for separation of the heteropoly compounds. Some problem still exist with these mobile phases. For example, an EtOAc/cyclohexane mixture was found as a good mobile phase in the SPSC system (C18 packing). Unfortunately, after the EtOAc/cyclohexane mixture was used on HPLC instrument for about two weeks, the instrument showed some strange phenomena. The column pressure was higher than 350 atm (the column pressure should not exceed 250 atm), and mobile phase leaked from some fittings. In this case, it is obvious that good results could not be obtained from the HPLC. One reason was that some materials of the C18 column were found to react with EtOAc (the end cups). This result illustrates that selection of solvents is very important.

A mixed mobile phase system, ACN/CH_2Cl_2, was found to be an alternative good solvent to elute the heteropoly compounds from the C18 SPCS system packing (see Table I). This mixture was used successfully as the mobile phase to separate the heteropoly compounds on HPLC (see Figure 21 and Table V). The 92/8 CH_2Cl_2/ACN mixture can be used to separate the AmCo+3B and the AmCo+2Si solutes. Since when the AmCo+3B and the AmCo+2Si solutes were separately injected onto HPLC at the same conditions (same mobile phase, same flow rate and same wavelength), some peaks appeared on the chromatogram when the AmCo+3B solute was injected; while in contrast when the AmCo+2Si solute was injected, nothing appeared on the chromatogram. This means that the AmCo+2Si and AmCo+3B can be separated using 92/8 CH_2Cl_2/ACN mixture.

Although 92/8 CH_2Cl_2/ACN mixture was found as a possible mobile phase to separate AmCo+3B and AmCo+2Si solutes, the fact that no peaks appeared on the chromatogram of AmCo+2Si was still a problem. This implied that the AmCo+2Si was still left in the column. A strong polarity solvent was needed to elute it. This would require a solvent program mode to finish the separation of the samples. So 92/8 CH_2Cl_2/ACN mixture was not a good mobile phase for an isocratic separation. Some other compositions of CH_2Cl_2/ACN were investigated further to separate heteropoly compounds, such as 90/10 CH_2Cl_2/ACN and 91/9 CH_2Cl_2/ACN. Unfortunately, peaks for AmCo+3B and AmCo+2Si appeared on the chromatographic chart in both mobile phases, and the retention times of these peaks were almost same.

Similar results were obtained with the silica column using 100% methylene chloride and 80/20 CH_2Cl_2/ACN mobile phases (see Table III and Figure 6 and Figure 9). The AmCo+2Si solute eluted from the silica column, while the AmCo+3B did not elute. This kind of problem need to be studied further when solvent programming capabilities are available.

In summary, mutual separation between AmCo+3B and AmCo+2Si has been achieved for the first time. Reversed-phase C18 liquid chromatography, with 66.7/33.3 MeOH/petroleum ether phosphate buffer was used as the eluent. From the results of the research, some factors were found to affect the separation of heteropoly. First, the more polar the solvent, the less the retention time will be. Heteropoly compounds can be separated with a certain polarity of the mobile phase. This result appears to be related to the polarity of the solvents. Second, the oxidation number of the cobalt was found to affect the separation. The less the oxidation number of the peripheral hetero atom, the longer the retention will be. The retention time of AmCo+3B was 22.5 min; and the retention time of AmCo+2Si was 31.5 min (see Table V and VI). In addition, as the "pH value" of the phosphate buffer of the 66.7/33.3 MeOH/petroleum ether mixture in the elute increases, the retention times of AmCo+2Si and AmCo+3B decreases (see Table V). Good chromatographic peaks were obtained when the "pH value" of the buffer was 4.5.

Presently, ion-pair chromatography generally is utilized for inorganic ions in reverse-phase chromatography. The results described above demonstrate that excellent separation of AmCo+3B and AmCo+2Si solutes can be achieved by reversed-phase C18 chromatography directly with the mixture of methanol and petroleum ether in phosphate buffer ("pH"=4.5). This was first time that this kind of 1:1:11 heteropoly samples (same charge of heteropoly anions joined with tetra-heptylammonium) were separated by HPLC. Moreover, these heteropoly samples (AmCo+3B and AmCo+2Si) used in the research were representive heteropoly compounds. So more heteropoly compounds can be expected to be separated by the mixture of methanol and petroleum ether mobile phase in the reversed-phase C18 liquid chromatography. We believe that there is an enormous potential for future development of this method. It is also possible that column materials such as SAX, SCX and Diol may be even better, as they do not have the "pH" = 3 - 8 limitations of C18 column. The separation of more heteropoly compounds and finding a versatile preparative HPLC method are worthy future goals. Also, the recovery of more highly charged polyoxometalates from the eluent components should be possible by the choice of "pH value", countercation, and solvents. An important feature of the present HPLC method is that the method provides a fast relatively low-cost monitoring technique for use in the separation of the heteropoly compounds.

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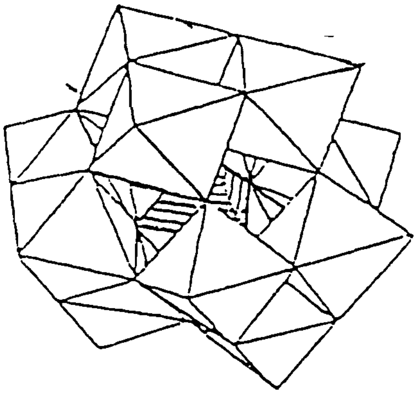
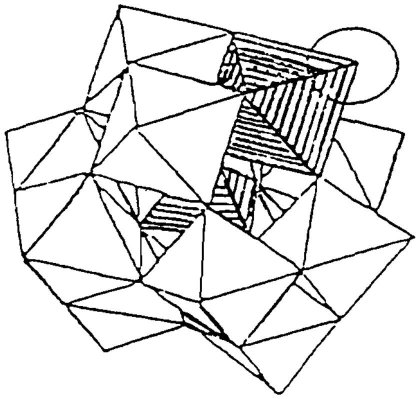
VI. APPENDIX A - FIGURES

Figure 1.

Keggin Structure



a



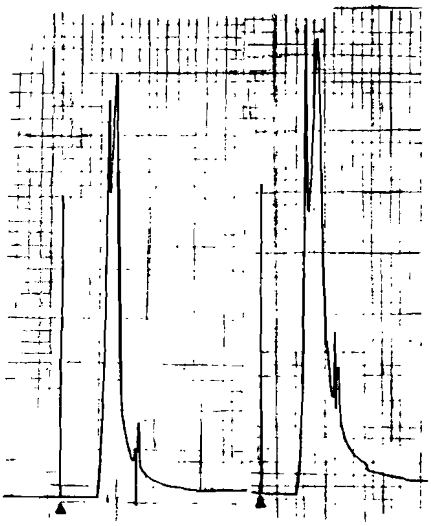
b

Figure 2.

Chromatograms of AmCo+3B and AmCo+2Si
in 100% Acetonitrile Using a Silica Column (first time)

(Small triangles below the chromatograms
identify the injection marks.
the ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

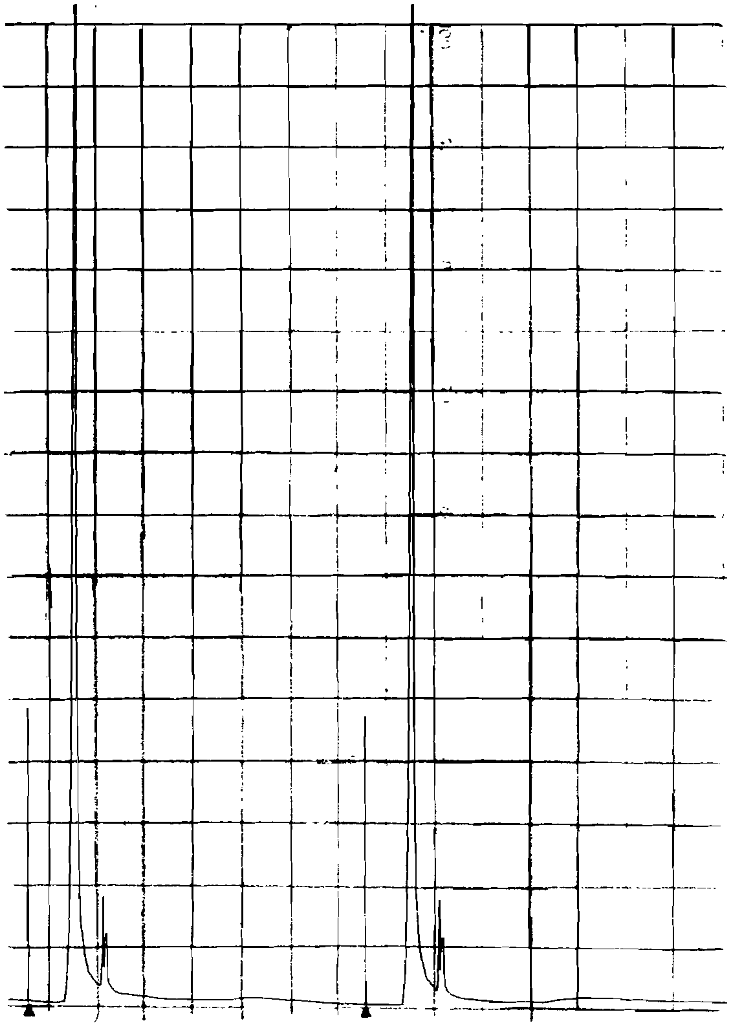
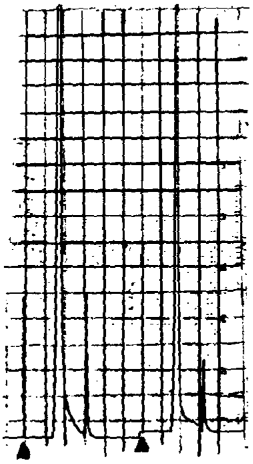


Figure 3.

Chromatograms of AmCo+3B and AmCo+2Si
in 100% Acetonitrile Using a Silica Column (second time)

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

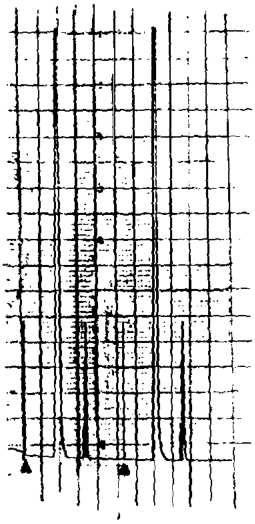
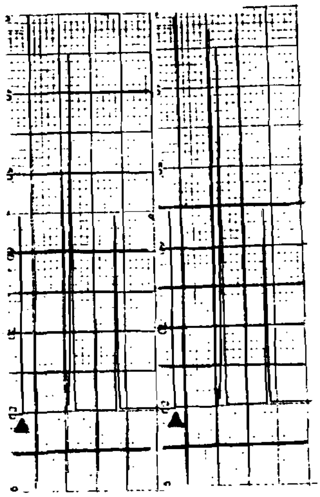


Figure 4.

Chromatograms of AmCo+3B and AmCo+2Si
in 100% Acetonitrile Using a Silica Column (third time)

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

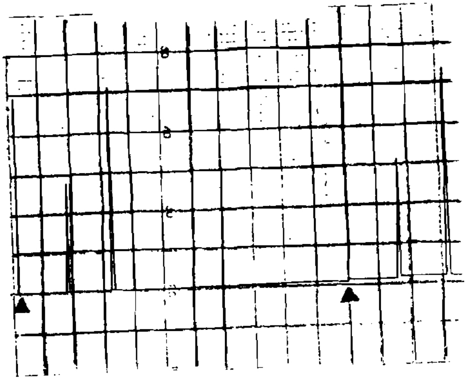
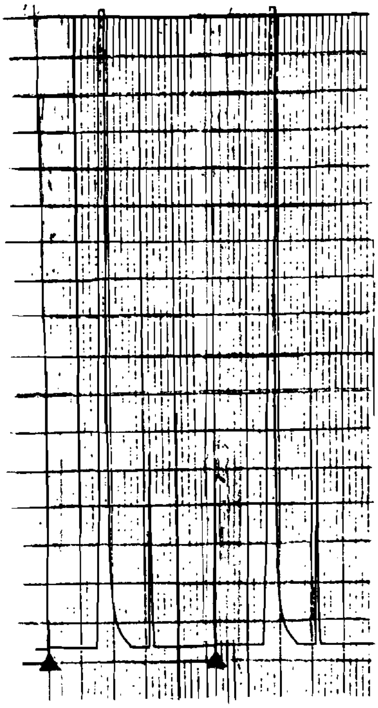


Figure 5.

Chromatograms of AmCo+3B and AmCo+2Si
in 100% Acetonitrile using a Silica Column
(Sample Including 2 Drops Ethyl Acetate)

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

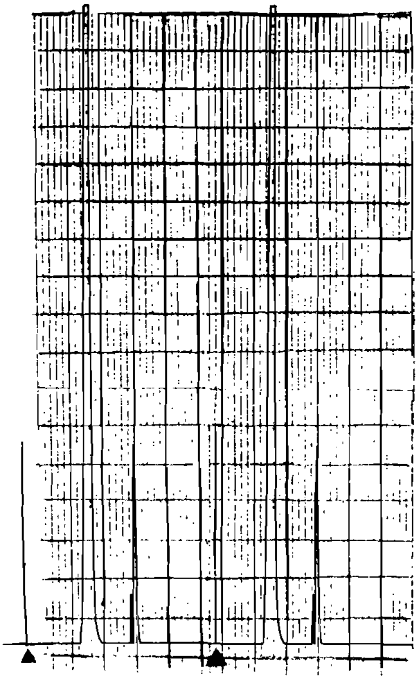
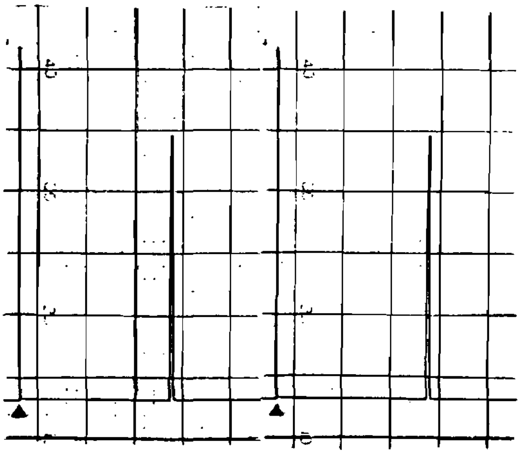


Figure 6.

Chromatograms of AmCo+3B and AmCo+2Si in 100% Methylene Chloride Using a Silica Column

(Small triangles below the chromatograms identify the injection marks.
The ordinate (y-axis is relative absorbance, the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

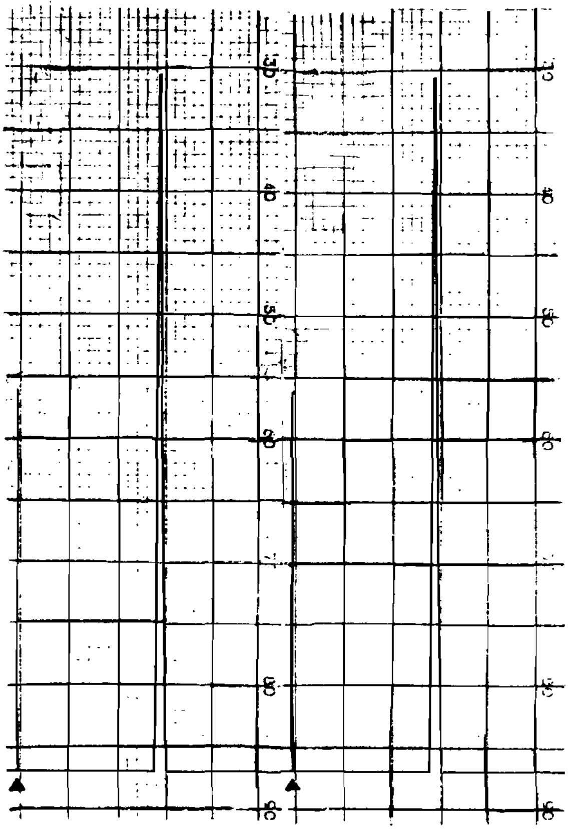
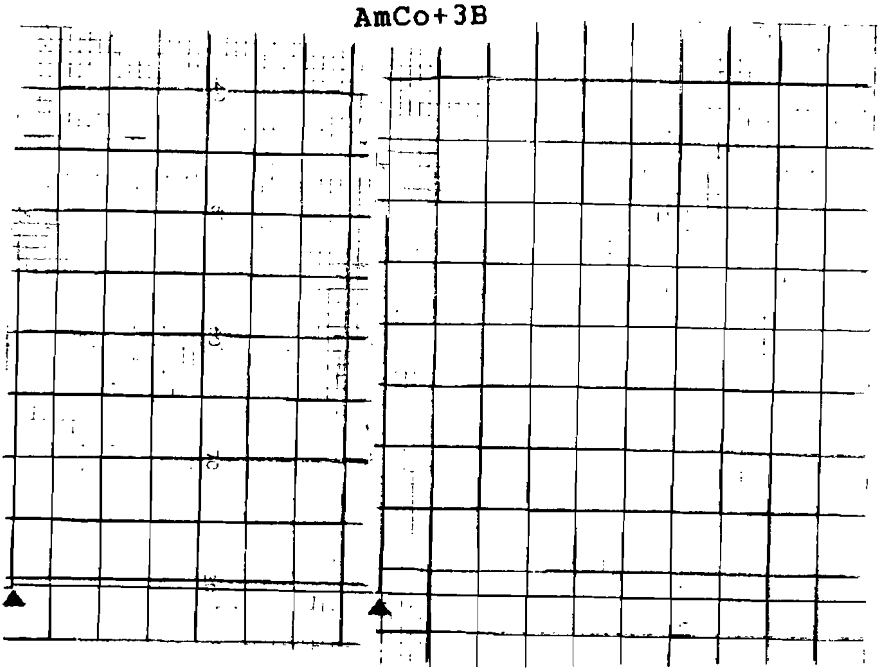


Figure 7.

Chromatograms of AmCo+3B and AmCo+2Si
in 95/5 CH_2Cl_2/ACN Mixture Using a Silica Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

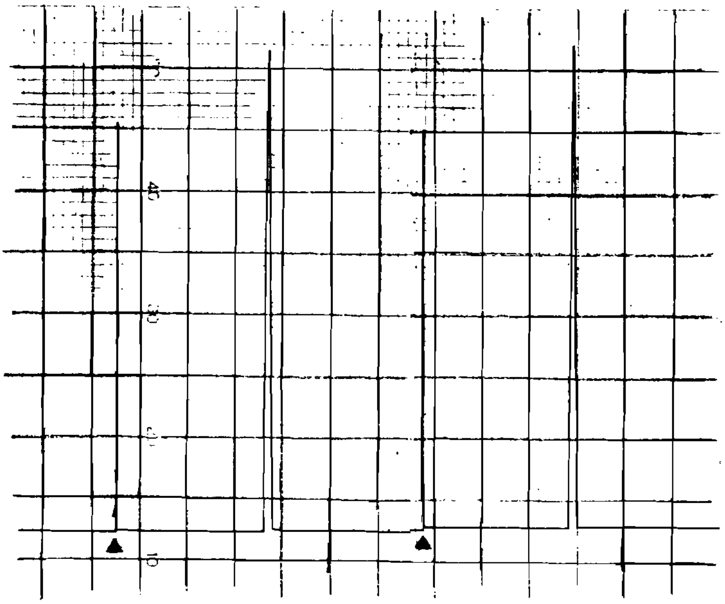
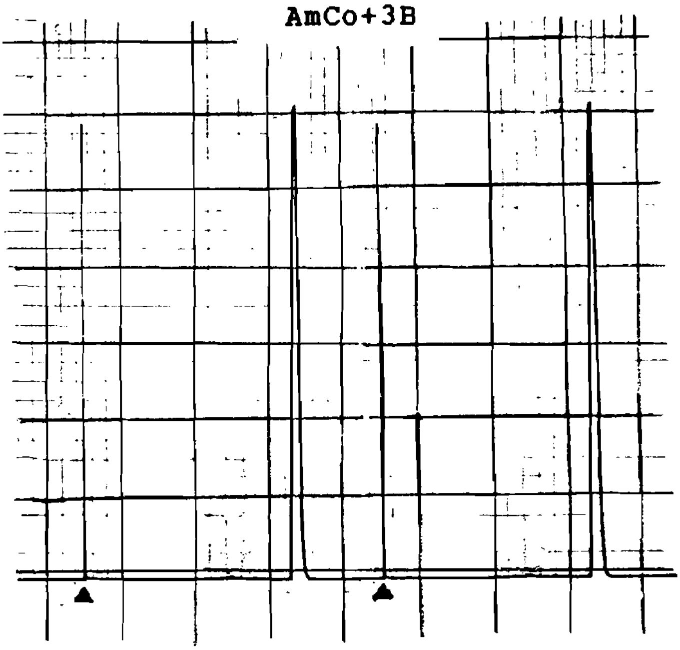


Figure 8.

Chromatograms of AmCo+3B and AmCo+2Si
in 90/10 CH_2Cl_2/ACN Mixture Using a Silica Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

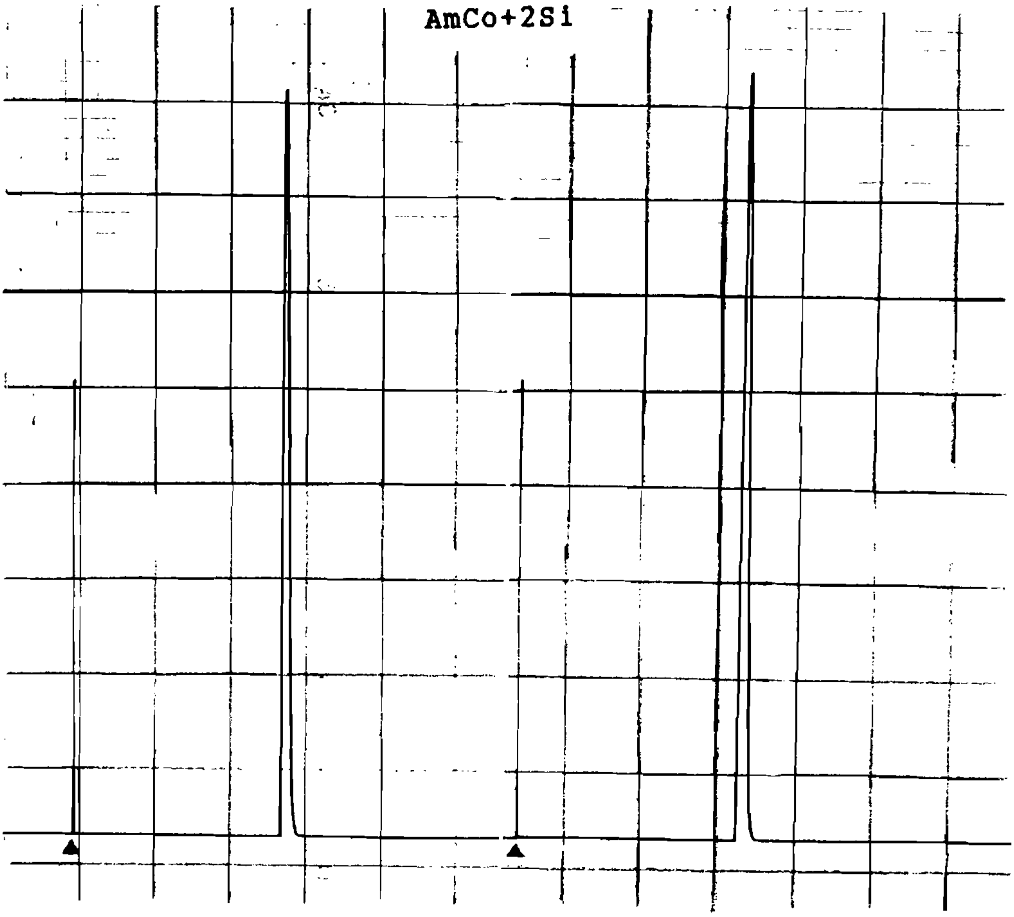
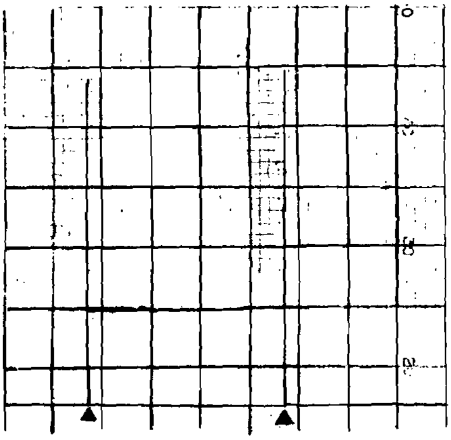


Figure 9.

Chromatograms of AmCo+3B and AmCo+2Si
in 80/20 Ch_2Cl_2/ACN Mixture Using a Silica Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

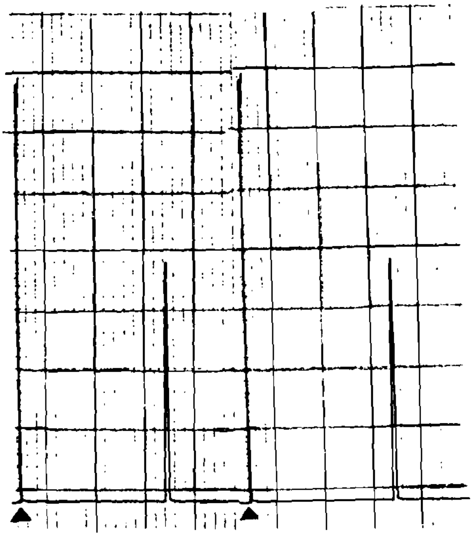
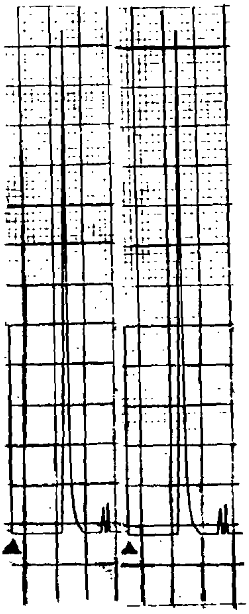


Figure 10.

Chromatograms of AmCo+3B and AmCo+2Si
in 50/50 CH_2Cl_2/ACN Mixture Using a Silica Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

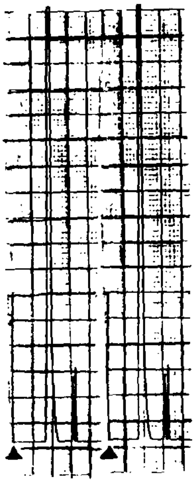
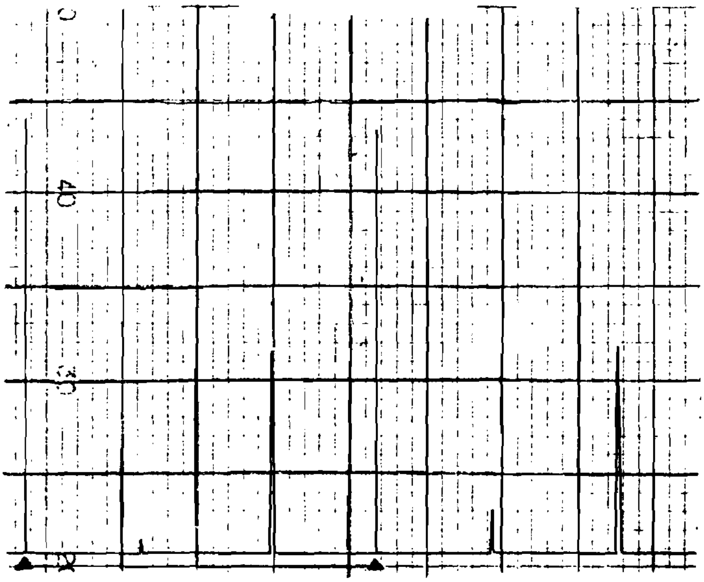


Figure 11.

Chromatograms of AmCo+3B and AmCo+2Si
in 45/55 CH_2Cl_2/ACN Mixture Using a Silica Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

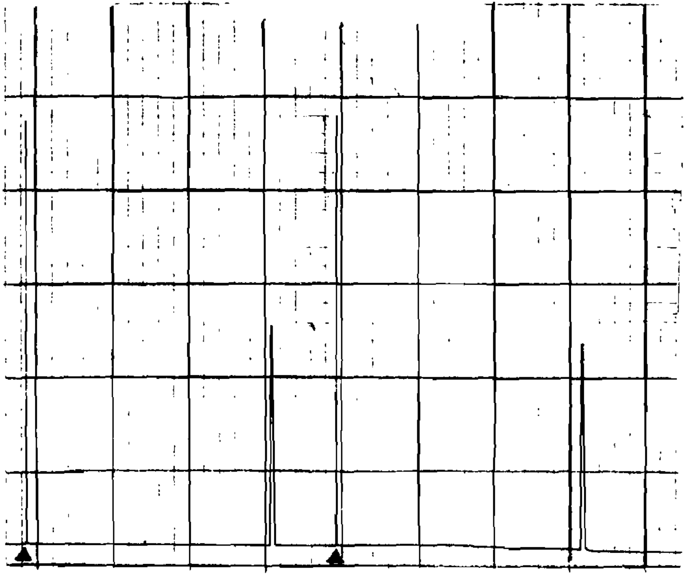
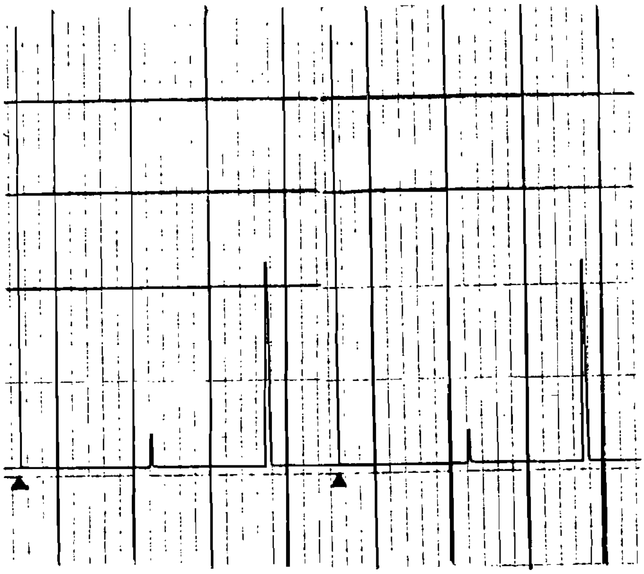


Figure 12.

Chromatograms of AmCo+3B and AmCo+2Si
in 35/65 CH_2Cl_2/ACN Mixture Using a Silica Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

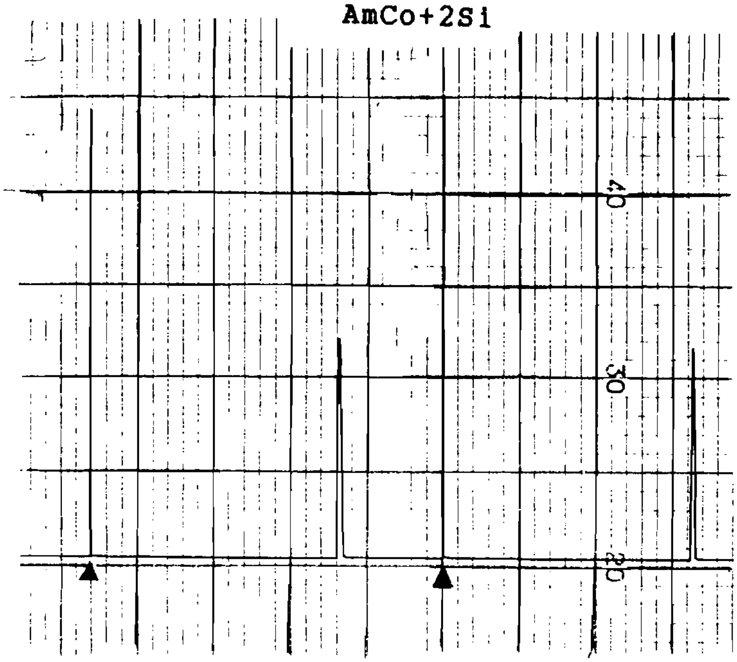
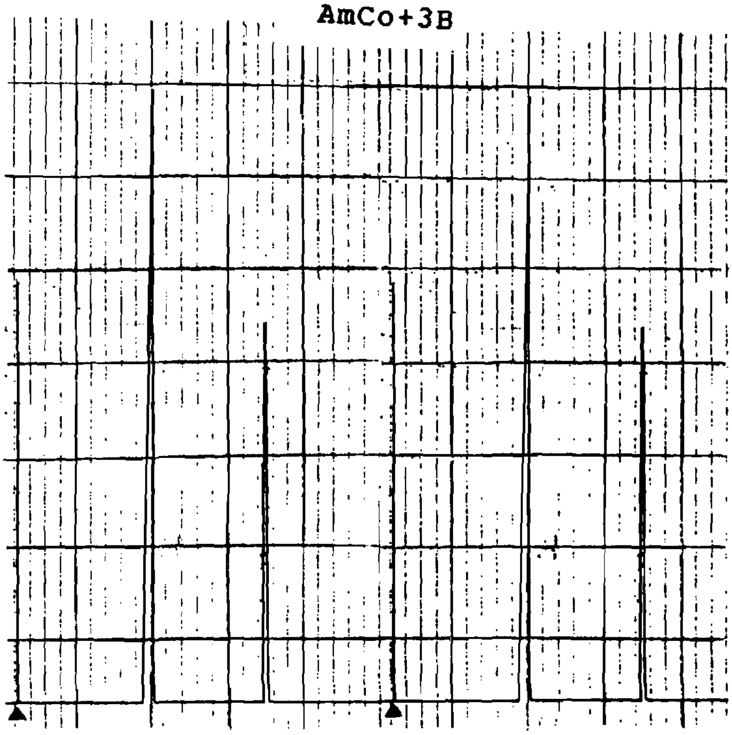


Figure 13.

Chromatograms of AmCo+3B and AmCo+2Si
in 32/68 CH_2Cl_2/ACN Mixture Using a Silica Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

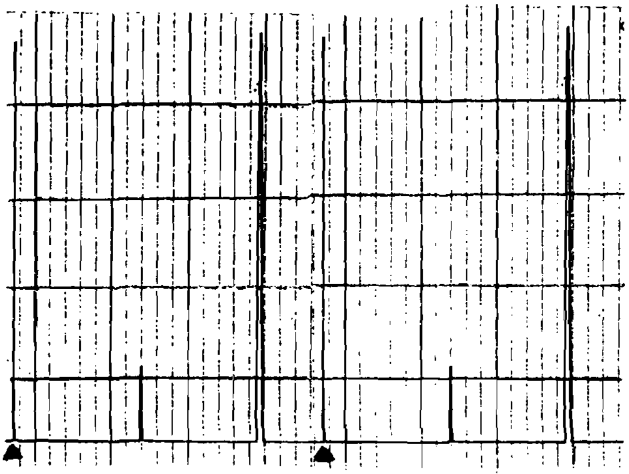
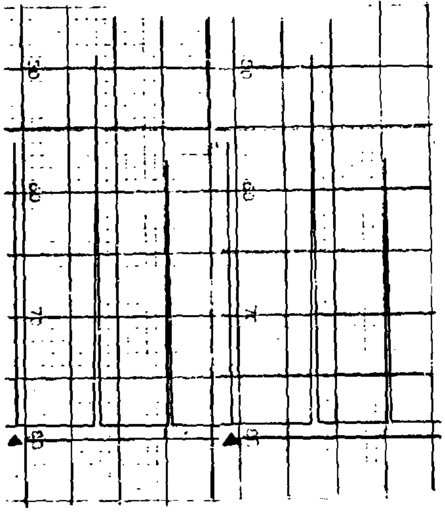


Figure 14.

Chromatograms of AmCo+3B and AmCo+2Si
in 27/73 CH_2Cl_2/ACN Mixture Using a Silica Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

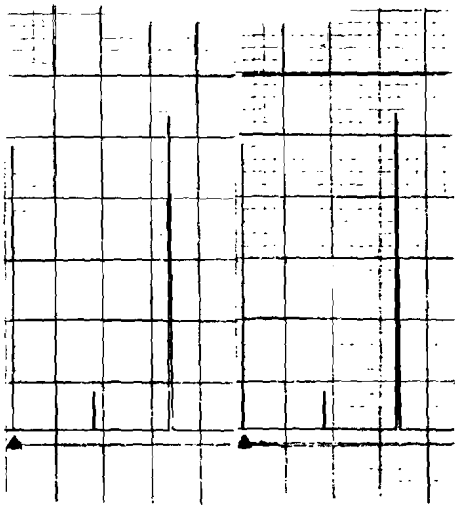
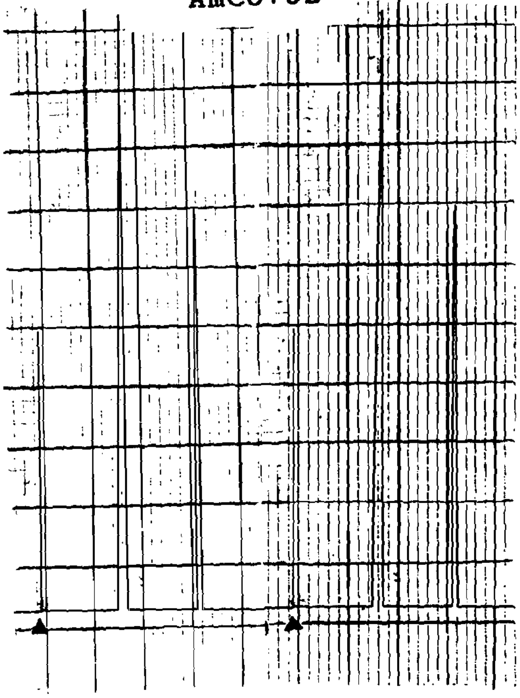


Figure 15.

Chromatograms of AmCo+3B and AmCo+2Si
in 23/77 CH_2Cl_2/ACN Mixture Using a Silica Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

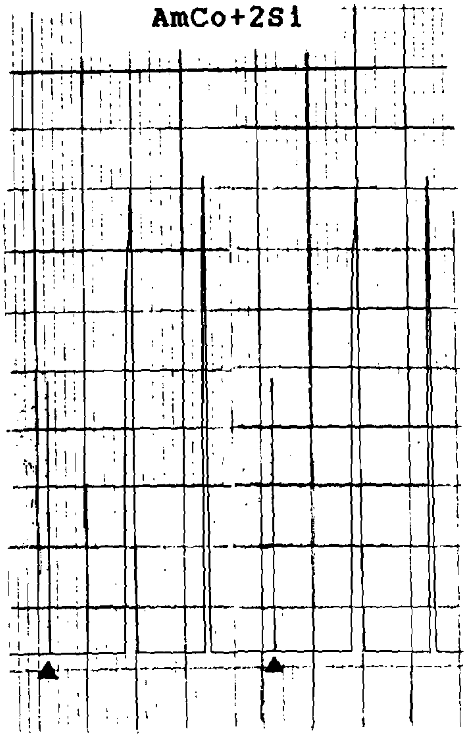


Figure 16.

Chromatograms of AmCo+3B and AmCo+2Si
in 50/50 EtOAc/Cyclohexane Mixture Using a C18 Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

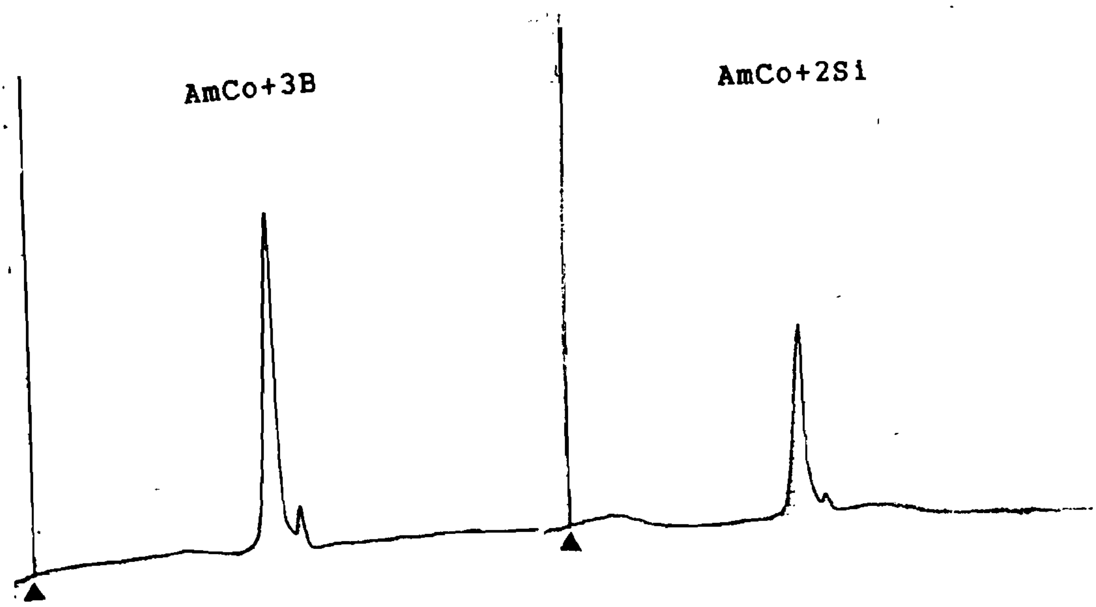

AmCo+3B
AmCo+2Si

Figure 17.

Chromatograms of AmCo+3B and AmCo+2Si
in 80/20 EtOAc/Cyclohexane Mixture Using a C18 Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

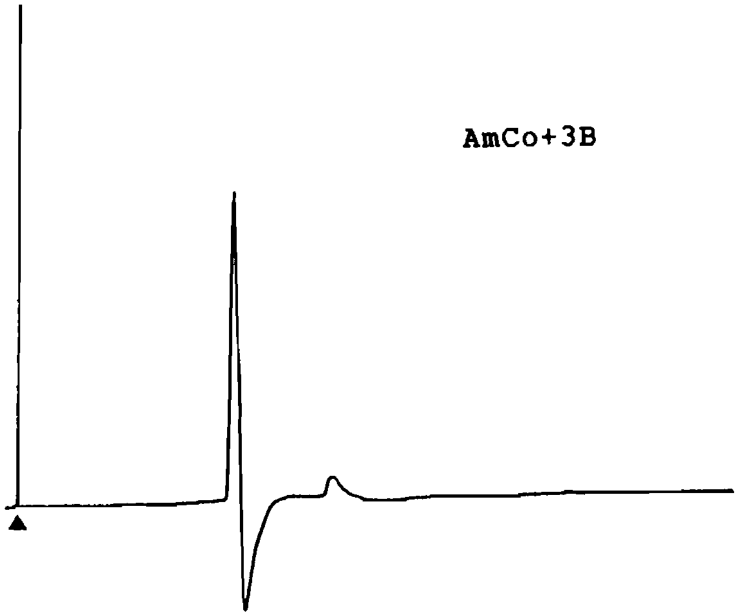
AmCo+3B

AmCo+2Si

Figure 18.

Chromatograms of AmCo+3B and AmCo+2Si
in 90/10 EtOAc/Cyclohexane Mixture Using a C18 Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)


AmCo+3B

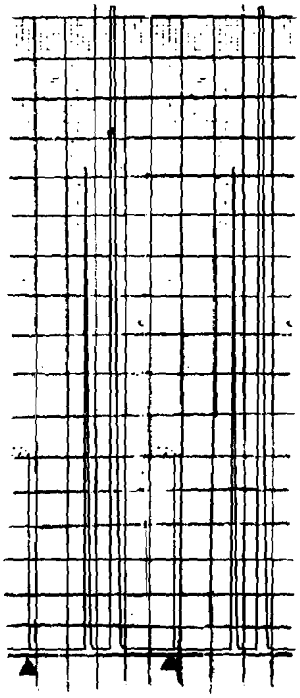

AmCo+2Si

Figure 19.

Chromatograms of AmCo+3B and AmCo+2Si
in 80/20 CH_2Cl_2/ACN Mixture Using a C18 Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

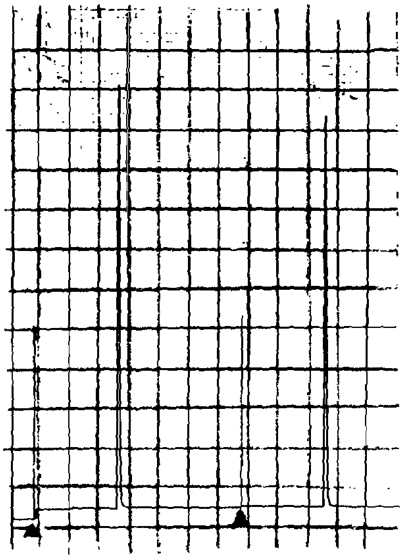
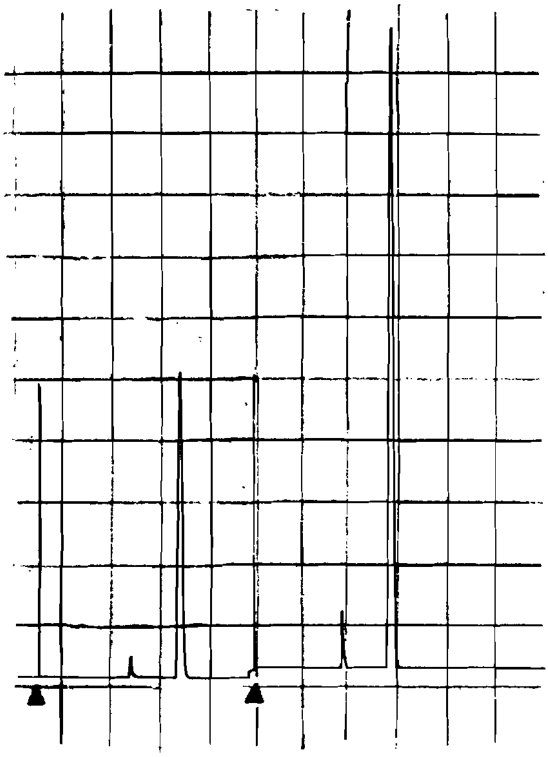


Figure 20.

Chromatograms of AmCo+3B and AmCo+2Si
in 90/10 CH_2Cl_2/ACN Mixture Using a C18 Column

(Small triangles below the chromatograms
identify the injection marks.
The abscissa (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)

AmCo+3B



AmCo+2Si

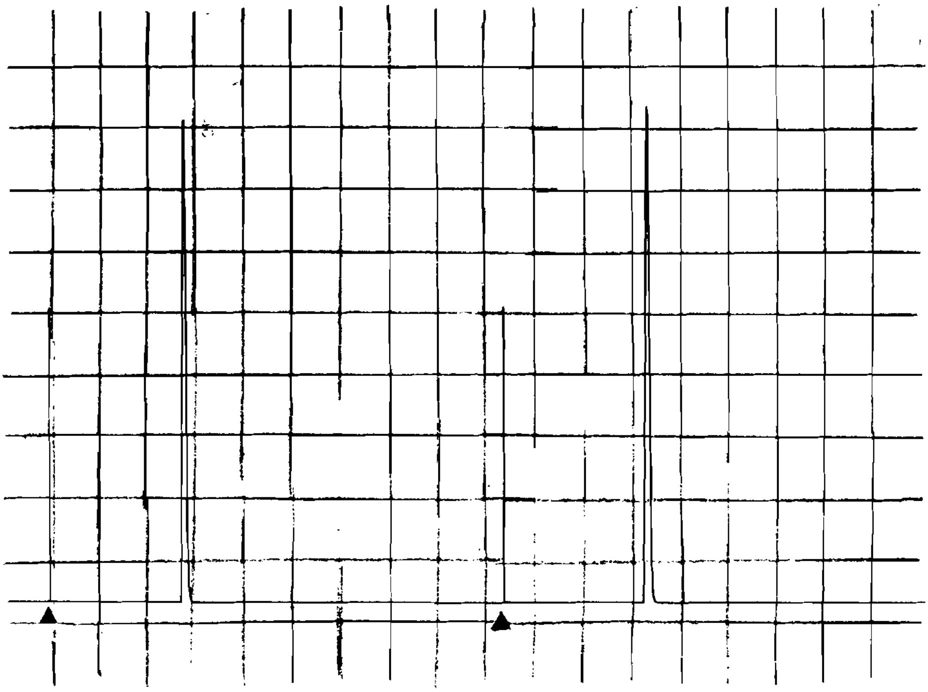
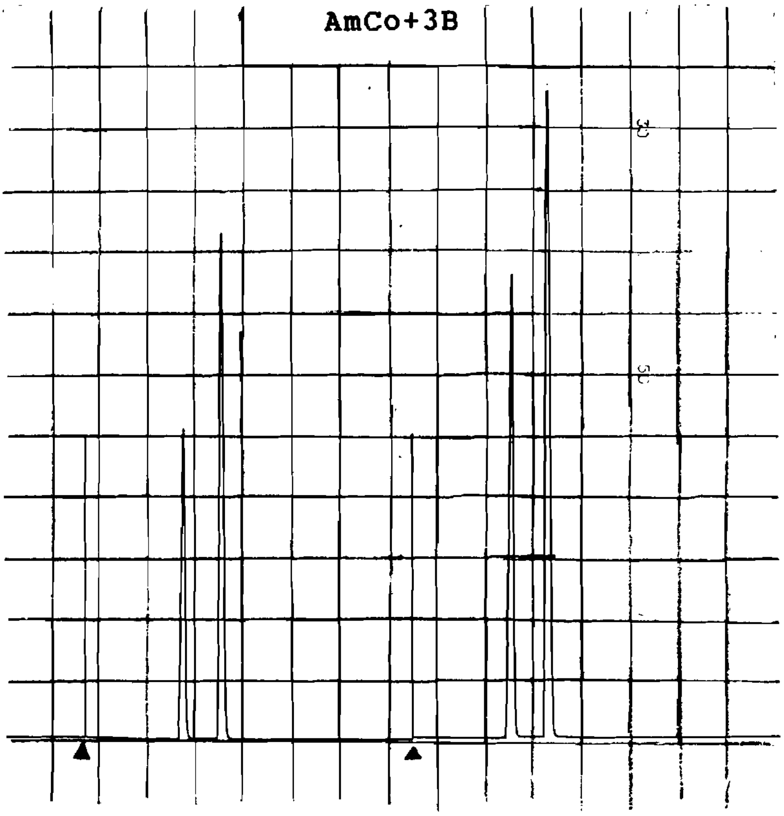


Figure 21.

Chromatograms of AmCo+3B and AmCo+2Si
in 92/8 CH_2Cl_2/ACN Mixture Using a C18 Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=1.0 ml)


AmCo+3B

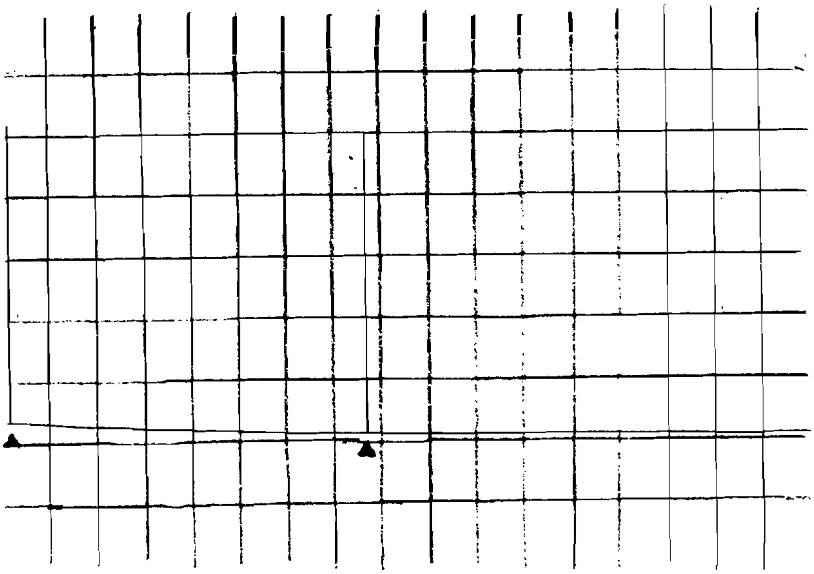
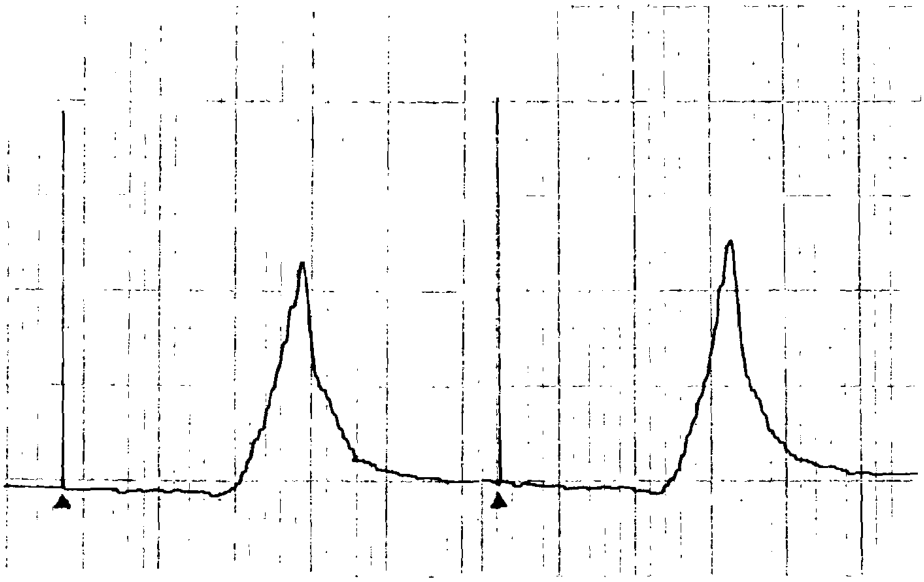

AmCo+2Si

Figure 22.

Chromatograms of AmCo+3B and AmCo+2Si
in 50/50 MeOH/Petro. Ether Mixture Using a C18 Column

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=2.0 ml)

AmCo+3B



AmCo+2Si

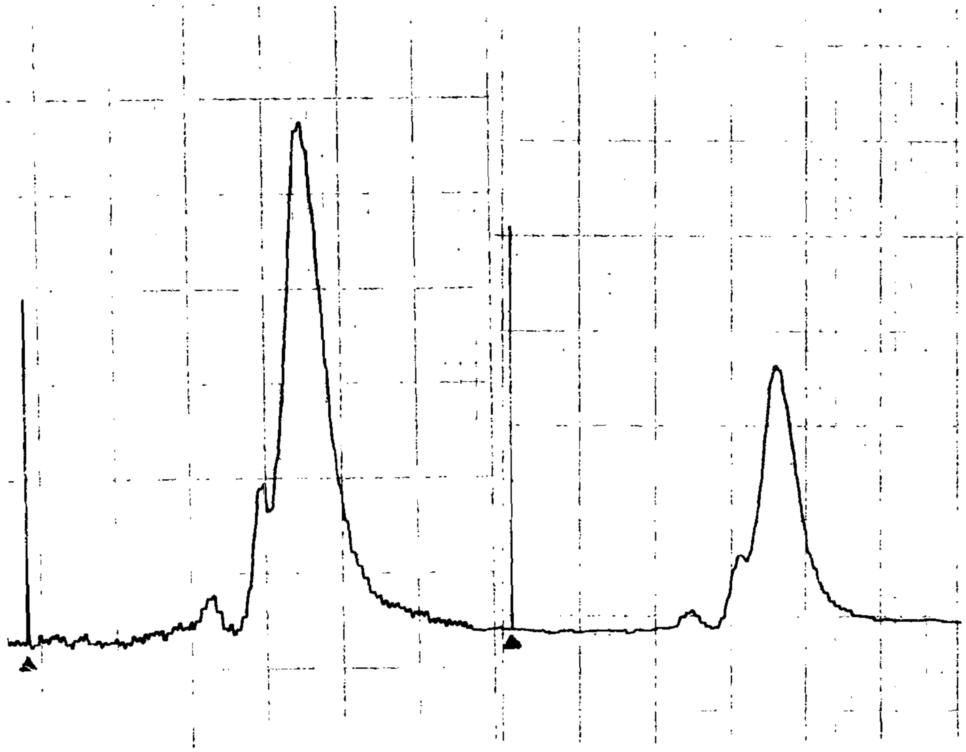
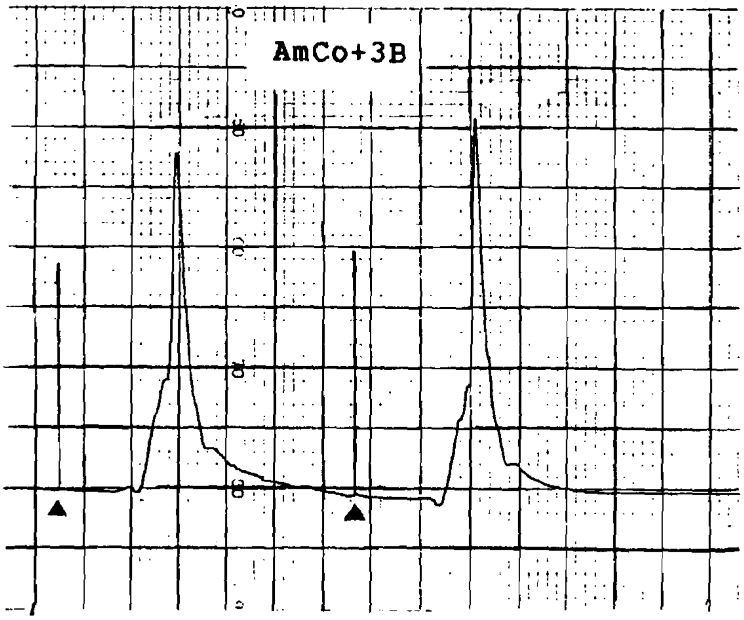


Figure 23.

Chromatograms of AmCo+3B and AmCo+2Si
in 66.7/33.3 MeOH/Petro. Ether Mixture Using a C18 Column
("pH'=7.3)

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=2.0 ml)


AmCo+3B

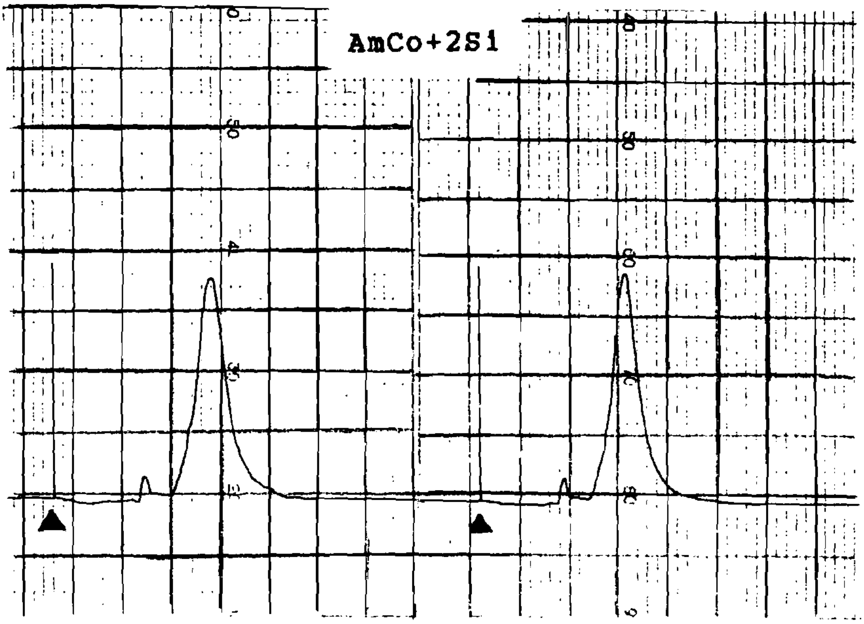

AmCo+2S1

Figure 24.

Chromatograms of Mixture of AmCo+3B and AmCo+2Si in 66.7/33.3 MeOH/Petro. Ether Mixture Using a C18 Column ("pH"=7.3)

(Small triangles below the chromatograms identify the injection marks.
The ordinate (y-axis) is relative absorbance units, the abscissa (x-axis) distance is time or volume.
1.0 cm=2.0 ml)

Mixture of AmCo+2Si and AmCo+3B

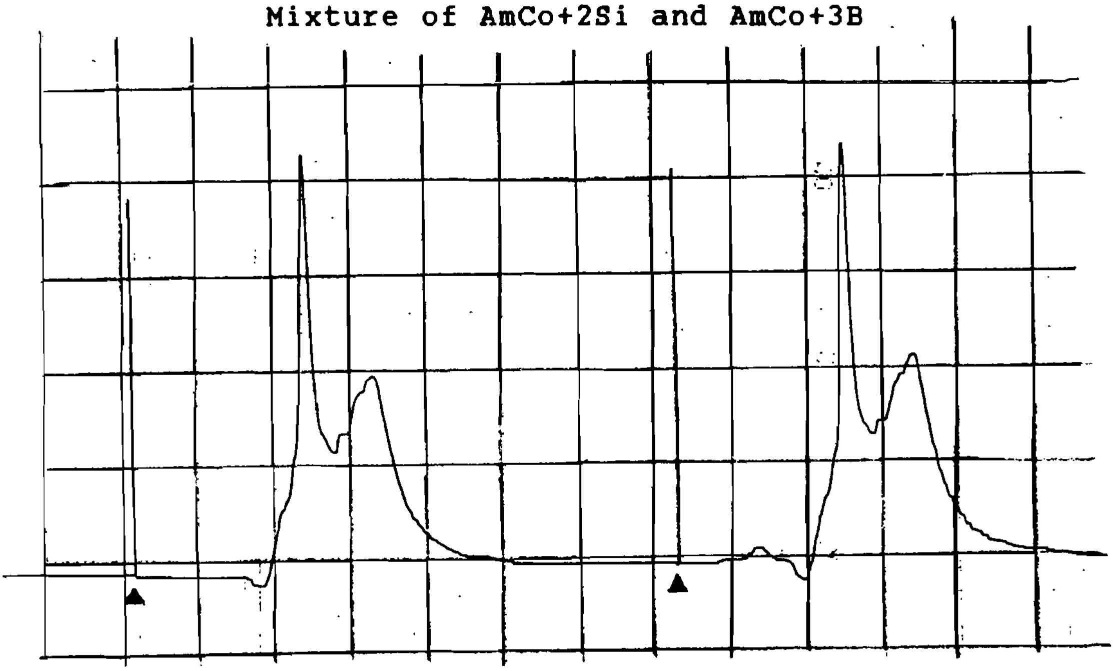
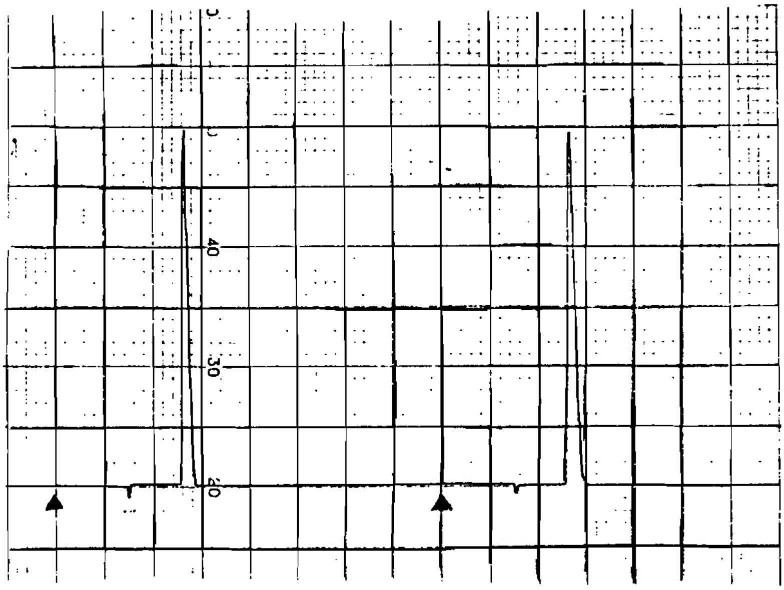


Figure 25.

Chromatograms of AmCo+3B and AmCo+2Si
in 66.7/33.3 MeOH/Petro. Ether Mixture Using a C18 Column
("pH"=4.5)

(Small triangles below the chromatograms
identify the injection marks.
The ordinate (y-axis) is relative absorbance units,
the abscissa (x-axis) distance is time or volume.
1.0 cm=2.0 ml)

AmCo+3B



AmCo+2Si

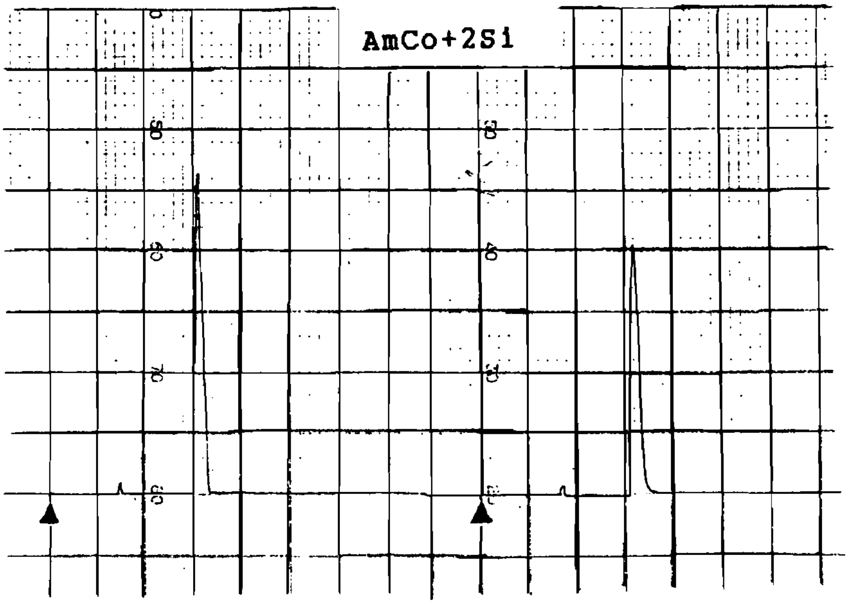
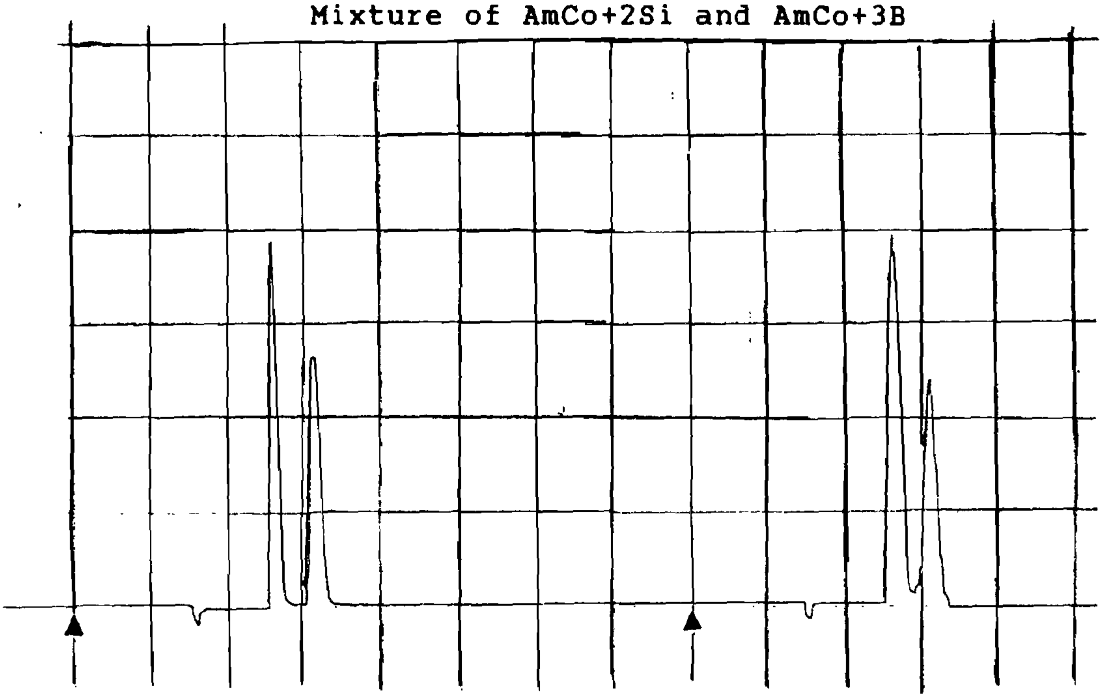


Figure 26.

Chromatograms of Mixture of AmCo+3B and AmCo+2Si in 66.7/33.3 MeOH/Petro. Ether Mixture Using a C18 Column ("pH"=4.5)

(Small triangles below the chromatograms identify the injection marks.
The ordinate (y-axis) is relative absorbance units, the abscissa (x-axis) distance is time or volume.
1.0 cm=2.0 ml)

Mixture of AmCo+2Si and AmCo+3B



VII. APPENDIX B - TABLES

Table I

Elution of AmCo+3B and AmCo+2Si in Various Mobile Phases in SPCS System (C18 Packing)

Solvent	Red AmCo+2Si	Green AmCo+3B
Cyclohexane	not elute	not elute
CCl_4	not elute	not elute
Toluene	not elute	not elute
CH_2Cl_2	not elute	not elute
90% EtOAc 10% Petro. ether	elute	elute
80% EtOAc 20% Petro. ether	part of prod. elute	part of prod. elute
90% EtOAc 10% Cyclohexane	elute	elute
80% EtOAc 20% Cyclohexane	elute	elute
50% EtOAc 50% Cyclohexane	elute	elute
MeOH	elute	elute
80% CH_2Cl_2 & 20% MeOH	elute	elute
90% CH_2Cl_2 & 10% MeOH	elute	elute
100% ACN	elute	elute
50% CH_2Cl_2 & 50% ACN	elute	elute
80% CH_2Cl_2 & 20% ACN	elute	elute
90% CH_2Cl_2 & 10% ACN	elute	elute
50% MeOH 50% Petro. ether	elute	elute
66.7% MeOH 33.3% Petro. ether	elute	elute

Table II

Elution of AmCo+3B and AmCo+2Si in Various Mobile Phases in Various SPCS Systems

Column (Packing)	Solvent	Red AmCo+2Si	Green AmCo+3B
Silica	Cyclohexane	not elute	not elute
	CH_2Cl_2	elute	elute
	ACN	elute	elute
	90% EtOAc 10% Cyclohexane	elute	elute
	50% EtOAc 50% Cyclohexane	elute	elute
	90% ACN & 10% CH_2Cl_2	elute	elute
	80% ACN & 20% CH_2Cl_2	elute	elute
	50% ACN & 50% CH_2Cl_2	elute	elute
	10% ACN & 90% CH_2Cl_2	elute	elute
	5% ACN & 95% CH_2Cl_2	elute	part of prod. elute
	2% ACN & 98% CH_2Cl_2	elute	part of prod. elute
	50% ACN & 50% H_2O	elute turbid	elute
Diol	CH_2Cl_2	elute	elute
	80% CH_2Cl_2 & 20% ACN	elute	elute
	80% CH_2Cl_2 20% Cyclohexane	elute	elute
	Cyclohexane	elute	elute
NH_2 (Amino)	80% CH_2Cl_2 20% Cyclohexane	elute	elute
SAX (Anion Exchange)	CH_2Cl_2	elute	elute
SCX (Cation Exchange)	CH_2Cl_2	elute	elute

Table III

Retention Times of AmCo+3B and AmCo+2Si in Different Mobile Phases in the Silica Column

Mobile Phase	AmCo+3B (T_r/mm)				AmCo+2Si (T_r/mm)		
	pk 1	pk 2	pk 3	pk 4	pk 1	pk 2	pk 3
100% ACN (1st time)	9.5	12	15	16	9.7	15.5	16
	9.8	11	15.5	16.3	9.8	15.5	16
100% ACN (2nd time)	18.5	30	31.7	none	18	30.5	32
	19	30.9	32.2	none	18	30.6	31.9
100% ACN (3rd time)	15	18.5	31	none	16.8	31.5	none
	14.9	18.5	31	none	16.5	31.5	none
100% ACN & 2 drops EtOAc in Sample	18.2	31.8	none	none	18	30.5	32
100% CH_2Cl_2	none	none	none	none	32	none	none
	none	none	none	none	31.5	none	none
95/5 CH_2Cl_2/ACN	31	none	none	none	31	none	none
	31.5	none	none	none	31.5	none	none
90/10 CH_2Cl_2/ACN	30.5	none	none	none	30.5	none	none
80/20 CH_2Cl_2/ACN	none	none	none	none	30	none	none
	none	none	none	none	30	none	none
50/50 CH_2Cl_2/ACN	18.5	29.8	34	none	18.5	30.5	32.5
	18.6	29.9	34	none	19	30	32.5
45/55 CH_2Cl_2/ACN	15	32	none	none	32	none	none
	15.2	32	none	none	32	none	none
35/65 CH_2Cl_2/ACN	15	32.5	none	none	32.5	none	none
	15	32.3	none	none	32.5	none	none
32/68 CH_2Cl_2/ACN	17.5	32.5	none	none	17.1	32.6	none
	17.4	32.6	none	none	16.8	32.1	none
27/73 CH_2Cl_2/ACN	17.5	32.5	none	none	16.5	32	none
23/77 CH_2Cl_2/ACN	17.8	32.5	none	none	17	32	none

Table IV

Refractive Indices of the Mixtures of Methylene Chloride and Acetonitrile

ACN%	Refractive Index 25.5°C	Experimental RI	Calculated ACN% (C)
0	1.4219	1.4155	8.1
20	1.4060	1.3740	60.4
40	1.3902	1.3755	58.5
50	1.3823	1.3708	64.4
60	1.3743	1.3683	67.6
80	1.3585	1.3643	72.6
100	1.3426	1.3601	77.0
Equation		RI = -0.0793*C + 1.4219	

```
                Regression Output:
Constant                           1.4219
Std Err of Y Est                 1.99E-16
R Squared                               1
No. of Observations                     7
Degrees of Freedom                      5

X Coefficient(s)   -0.0793
Std Err of Coef.  2.37E-16
```

Table V

Retention Times of AmCo+3B and AmCo+2Si in Different Mobile Phases in the C18 Column

Mobile phase	AmCo+3B (T_r/mm)			AmCo+2Si (T_r/mm)		
	pk 1	pk 2	pk 3	pk 1	pk 2	pk 3
50/50 EtOAc/Cyclohexane	30.5	34	none	31.5	35	none
80/20 EtOAc/Cyclohexane	31	33.6	none	31	34.5	none
90/10 EtOAc/Cyclohexane	29	31	42.3	29.5	31.5	43.5
80/20 CH_2Cl_2/ACN	19.5	28	29.5	20	29	none
	19.6	28	29.7	20	29.5	none
90/10 CH_2Cl_2/ACN	18.8	28.3	none	28.3	none	none
	18.5	28.8	none	28.2	none	none
92/8 CH_2Cl_2/ACN	20.8	28.5	none	none	none	none
	20.6	28.4	none	none	none	none
50/50 MeOH/Petro. Ether	31.5	none	none	24	31	36.5
	31.1	none	none	23.7	30	36
66.7/33.3 MeOH/Petro. Ether ("pH"=7.3)	16	24.5	none	18	32	none
	16	24.5	none	17.9	31.8	none
66.7/33.3 MeOH/Petro. Ether ("pH"=4.5)	15	26.5	none	15	30.9	none
	15	24.6	none	15.2	31	none

Mobile Phase	Mixture of AmCo+3B and AmCo+2Si (T_r/mm)		
	pk 1	pk 2	pk 3
66.7/33.3 MeOH/Petro. Ether ("pH"=7.3)	16	22.5	31.5
	15.9	22	31.5
66.7/33.3 MeOH/Petro. Ether ("pH"=4.5)	16	25.5	31
	15.8	25.8	31

Table VI

Chromatographic Parameters of Mixture of AmCo+3B and AmCo+2 in Different "pH values" of the 66.7/33.3 MeOH/Petro. Ether Mobile Phase

The chart speed = 1.0 cm/min, T_o=1.60 min				
Mixture AmCo+3B & AmCo+2Si in 66.7/33.3 MeOH/petro. ether, "pH"=7.3				
Sample	AmCo+3B		AmCo+2Si	
	1st time	2nd time	1st time	2nd time
T_r/min	2.25	2.20	3.15	3.15
T'_r/min	0.65	0.60	1.55	1.55
$W_{1/2}$/min	0.48	0.47	0.88	0.90
W_b/min	0.82	0.80	1.50	1.53
N	122	121	71	68
k'	0.41	0.38	0.97	0.97
alpha	1st time		2nd time	
	2.38		2.58	
R	1st time		2nd time	
	0.78		0.81	
Mixture of AmCo+3B & AmCo+2Si in 66.7/33.3 MeOH/petro. ether,"pH"=4.5				
Sample	AmCo+3B		AmCo+2Si	
	1st time	2nd time	1st time	2nd time
T_r/min	2.55	2.58	3.10	3.10
T'_r/min	0.95	0.98	1.50	1.50
$W_{1/2}$/min	0.16	0.18	0.19	0.21
W_b/min	0.27	0.30	0.33	0.35
N	198	171	330	294
k'	0.59	0.61	0.94	0.94
alpha	1st time		2nd time	
	1.58		1.83	
R	1st time		2nd time	
	1.53		1.60	

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Gang Yuan
Signature of Author

Aug. 3, 1991
Date

INVESTIGATIONS INTO THE HPLC SEPARATION OF
TETRAALKYLAMMONIUM COBALTO-UNDECATUNGSTOMETALLATES
Title of Thesis

Jackie Tolbert
Signature of Graduate Office Staff Member

August 6, 1991
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