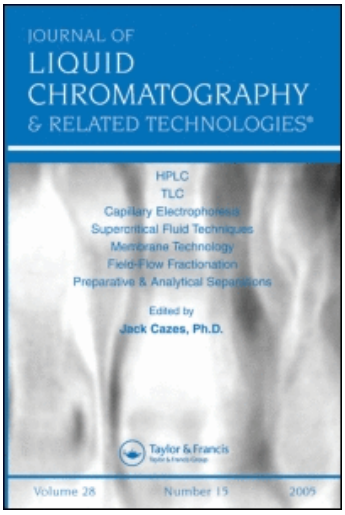

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Isolation and Identification of Drugs of Forensic Interest by High Performance Reverse Phase Ion Pair Partition Chromatography

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ISOLATION AND IDENTIFICATION OF DRUGS OF FORENSIC INTEREST BY HIGH PERFORMANCE REVERSE PHASE ION PAIR PARTITION CHROMATOGRAPHY

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ABSTRACT

A method is presented for the isolation and identification of milligram or microgram quantities of drugs of forensic interest. High performance reverse phase ion pair partition chromatography is performed on a 9.4 millimeter internal diameter microparticulate octadecylsilane column employing a water, methanol, acetic acid, alkylsulfonate mobile phase. Subsequent to collection from the liquid chromatograph, a simple extraction is performed followed by Infrared (IR) analysis and/or solid probe Mass Spectrometry (MS). A study is presented using phenylpropanolamine hydrochloride and ephedrine hydrochloride as a test model for the determination of the optimum concentration of counter ion required for a semi-preparative separation. The method is applied to an LSD seizure from a clandestine laboratory, methamphetamine in a street exhibit, and an amobarbital - secobarbital mixture in a multi-barbiturate capsule.

INTRODUCTION

For legal as well as intelligence purposes it is desirable to perform further analysis on the drugs whose peaks appear in an analytical chromatogram. One method would be to use a semi-prepar-

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ative column of similar packing material to an analytical column followed by Infrared or Mass Spectrometry. Although reverse phase ion pair partition chromatography is a most versatile analytical technique when applied to drugs of forensic interest[1], collection for further analysis is not recommended unless the interfering counter ion is removed[2].

This paper will cover the effect of using ion pairing reagents in a semi-preparative role and their subsequent removal for identification of drugs of forensic interest.

MATERIALS

Apparatus

The liquid chromatograph consisted of the following components: Model 6000A pump (Waters Associates, Milford, MA); Model U6K injector (Waters); a prepacked 4.4 mm. id. x 30 cm. stainless steel column, u-Bondapak C-18 (Waters) or a prepacked 9.4 mm. id. x 25 cm. stainless steel column, Magnum 9 Partisil-10 ODS (Whatman Inc., Clifton, NJ); Model 440 UV detector at 254 nm. and/or Model 401 RI detector (Waters) or Model 770 variable UV detector at 254 nm. (Schoeffel Instruments, Westwood, NJ); System IBV integrator (Spectra Physics, Santa Clara, CA).

A Model 3200 GC/MS interfaced to a Model 6110 data system (Finnigan Instruments, Sunnyvale, CA) was employed.

The Infrared Spectrophotometer utilized was Model 283 (Perkin Elmer Corporation, Norwalk, CT).

Chemicals

All mobile phases were prepared by dissolving an alkylsulfonic acid or alkylsulfonic salt (Eastman Chemicals, Rochester, NY) in a

solution consisting of glacial acetic acid, methanol, (Burdick and Jackson) and distilled water. After filtering and degassing the solution through a Millipore 0.45μ filter (Millipore Corp., Bedford, MA) the pH was adjusted to 3.5 with 2N NaOH.

The d-methamphetamine hydrochloride was obtained from Aldrich Chemicals, Milwaukee, WI. Ephedrine hydrochloride was obtained from Merck, Rahway, NJ, while the phenylpropanolamine hydrochloride was obtained from USP, Rockville, MD.

METHOD

The analytical profile of a sample in methanol is first determined on a u-Bondapak C-18 column. An analytical injection is then made on a Magnum 9 column with the same mobile phase as used on the analytical column except the counter ion concentration in most cases is scaled up from 0.005M to 0.04M. A concentrated extract of the sample is then dissolved in two milliliters of mobile phase and clarified through a .45 micron filter, type FLHOP1300 (Millipore) prior to an injection on the Magnum 9 column of approximately 1.4 ml.

Neutral drugs and those which exist as the free acid at pH 3.5 are extracted directly into chloroform two or three times after collection from the liquid chromatograph. Collected effluents containing basic drugs are adjusted with 1N NaOH to at least 1.5 pH units above the pKa value of the drug, if known,[3, 4] otherwise a pH of 11.5 is chosen.

RESULTS AND DISCUSSION

Effect Of Counter Ion On Semi-preparative Chromatography

A major consideration in going from an analytical to a semi-preparative column in reverse phase ion pair partition chromato-

graphy would be what concentration of counter ion to employ. Ion pair formation between a cationic basic salt and anionic counter ion occurs in the mobile phase and results in a lipophilic complex that is retained by a reverse phase column[5]. As sample size increases for a constant amount of counter ion, the ion pair reagent can become depleted resulting in lower k' values and increased tailing[1].

Since milligram amounts of sample are utilized on the Magnum 9 column, reduced k' values and increased tailing relative to microgram quantities of sample could be obtained in part due to insufficient counter ion. It was investigated whether this possible effect could be minimized by increasing the counter ion concentration. In this regard a study was performed whereby samples of five mcg. each and ten mg. each of phenylpropanolamine hydrochloride and ephedrine hydrochloride were injected onto a semi-preparative column at different counter ion concentrations with the ratio of water, methanol, and acetic acid kept constant. The percent reduction in k' for both drugs in going from five mcg. to ten mg. of each was measured. k' was calculated according to the formula $k' = (Vs - Vo)/Vo$ where Vs equals retention volume of sample peak and Vo is the retention volume for the methanol peak. A reduction in k' was obtained at all concentrations of counter ion. This was due to some extent to the column being in an overload condition; that is, k' values for sample peaks show a greater than 10% decrease as the sample weight increases[6]. For a silica absorbant more than one mg. of sample per gram of packing material represents an overload condition[6]. In our study we

have 20 mg. of sample in seven grams of a bonded silica packing which has a lower sample capacity than a silica packing Since there was some variation in percent k' reduction with counter ion concentration as Figure #1 shows, ion pairing seems to be playing a role. The reason for the difference in behavior for ephedrine versus phenylpropanolamine is not apparent at this time.

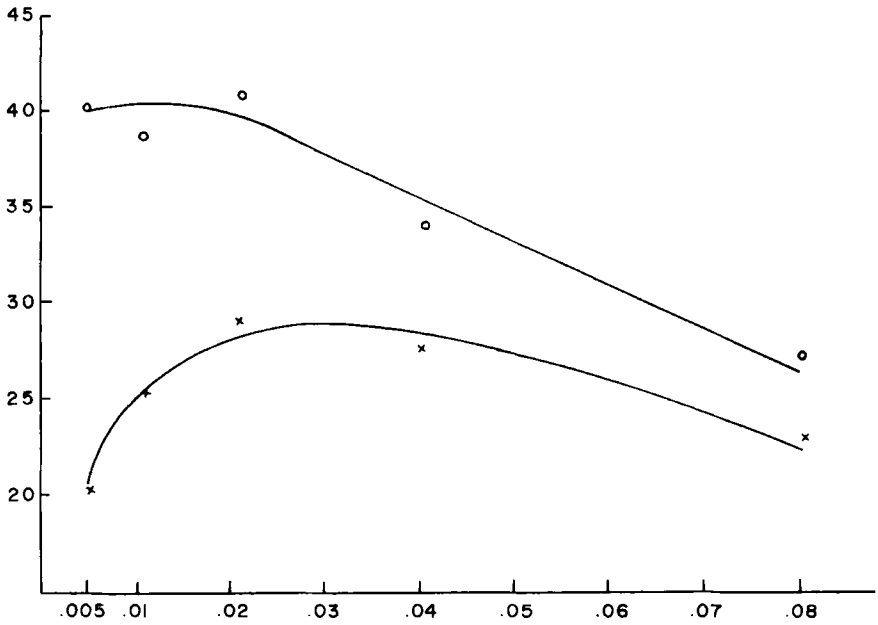

FIGURE 1

Plot of percent reduction of k' values from analytical to semi-preparative injection vs. counter ion concentration. For phenylpropanolamine hydrochloride (x's) and ephedrine hydrochloride (o's) the analytical injection consisted of 5 mcl. each of 0.5 mg/ml. methanolic solution while the semi-preparative injection consisted of 1.4 ml. of mobile phase containing 10 mg. of each component. Semi-preparative injections had precision of approximately 10%. Chromatograph conditions: Column, Magnum 9 ODS; fixed wavelength detector; mobile phase, water, methanol, acetic acid (79, 20, 1) with varying amounts of pentanesulfonic acid adjusted to pH 3.5 described in text; flow rate 5.0 ml/min.

The percent reduction in k' values in going from five mcg. ephedrine hydrochloride plus five mcg. phenylpropanolamine hydrochloride to ten mg. each on the Magnum 9 column was similar with either 0.04M heptanesulfonic acid or 0.04M methanesulfonic acid when the same water, methanol, acetic acid ratio as before was used.

In the above cases when milligram amounts of ephedrine hydrochloride and phenylpropanolamine hydrochloride were injected, significant tailing peaks of similar magnitude were observed most likely due to a combination of ion pair effects and column overload.

Since the variation above in percent reduction of k' values with counter ion concentration is relatively small it would appear that there are no significant differences in using a large or small counter ion concentration for the amounts of sample studied. However, this is not the case, since it was found that at a concentration below 0.04M pentanesulfonic acid, ephedrine and phenylpropanolamine will show peak splitting, see Figure #2. This phenomenon was confirmed by collecting various fractions, extracting and injecting them on an analytical column. The possible peaks that ephedrine and phenylpropanolamine split into may represent a pentanesulfonate complex, an acetate complex and uncomplexed material. Peak splitting was also observed for the two drugs with methanesulfonic acid and heptanesulfonic acid below a counter ion concentration of 0.04M. Thus to eliminate peak splitting, 0.04M counter ion concentration is recommended for the amount of sample illustrated.

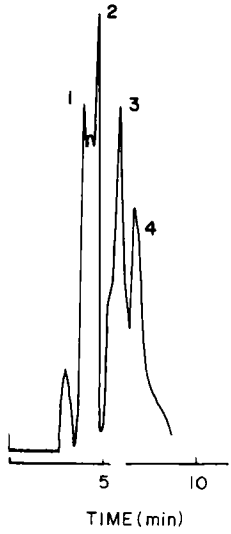

FIGURE 2

Semi-preparative chromatogram of 10 mg. ephedrine hydrochloride plus 10 mg. of phenylpropanolamine hydrochloride whose peaks represent: (1) phenylpropanolamine; (2) phenylpropanolamine plus ephedrine; (3) phenylpropanolamine plus ephedrine and (4) ephedrine. Conditions: Column, Magnum 9 ODS; fixed wavelength detector sensitivity 2.0 a.u.f.s.; mobile phase, water methanol, acetic acid (79, 20, 1) with 0.005M pentanesulfonic acid adjusted to pH 3.5 as described in text; flow rate 5.0 ml/min.

Elimination of Counter Ion

After collecting the peaks of interest from the liquid chromatograph it is necessary to remove the counter ion. At a pH value that is 1.5 units below its pKa value a basic drug will exist mostly as its catonic salt and the resulting complex with a counter ion could be extracted into chloroform. If the mobile phase was adjusted to a pH 1.5 units above the pKa value then predominately free base would partition into chloroform. This was experimentally observed for ephedrine which has a pKa value of 9.5. Ten mg. of ephedrine

hydrochloride were repeatedly injected onto a Magnum 9 column equilibrated with mobile phase containing 0.04M heptanesulfonic acid. The ephedrine peak was collected and extracted with chloroform directly and at integral pH values of 7 thru 12.

After extracting the ephedrine with chloroform and evaporated in the presence of concentrated hydrochloric acid, the extract in methanol was analyzed for ephedrine content, see Table 1. The data is consistent with a complex being extracted since at a pH of nine and below a smaller amount of ephedrine would be expected with virtually no ephedrine at pH 3.5.

TABLE 1

Calculated yields from 10 mg. injections on a Magnum 9 column of ephedrine hydrochloride extracted at various pH values from a mobile phase containing 20% methanol, 79% water, 1% acetic acid with 0.04M heptanesulfonic acid, at 3.5. Chromatography conditions for ephedrine assay: Column, u-Bondapak C-18; variable wavelength detector; mobile phase, water, methanol, acetic acid (79, 20, 1) with 0.005M propanesulfonic acid adjusted to pH 3.5 as described in text; flow rate, 2 ml/min.

pH	Milligram Ephedrine (calc. as HCl)
direct	7.4
7	6.7
8	6.8
9	8.1
10	8.6
11	7.3
12	7.6

The MS with data system was used to determine whether or not the heptanesulfonate complex was present in the extract. Complex was found to be present at pH 3.5, pH 7, pH 8, and pH 9 in decreasing amounts. A small amount of complex was found at pH 10 and none was found at pH 11 and pH 12.

Figure #3 depicts the total ion chromatogram of ephedrine extracted at pH 9. The total intensity of each scan is plotted versus the scan number. The identification of the presence of complex was determined through the use of a technique known as a "limited mass plot". This is done by asking the computer to search through a spectra file and locate those spectra which contain specific ion fragments. This specific mass search is used to determine

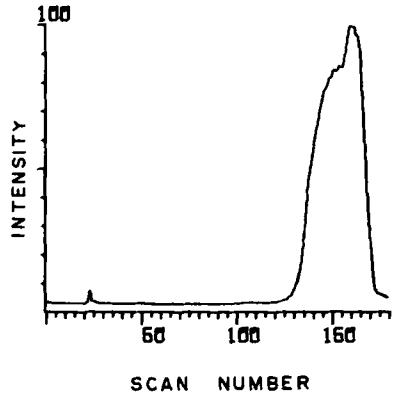

FIGURE 3

Total ion chromatogram of ephedrine extracted at pH 9 obtained via a direct insertion solid probe at 250-300°C under the following conditions: Electron Energy 70 ev, Filament Current 1 ma, Multiplier Gain 1.2 kv., Manifold Temperature 100°C. Other source potentials were adjusted to provide maximum sensitivity and resolution.

the presence of a compound in an unresolved peak. Any of the 180 spectra collected may be recalled to give a mass spectrum at that point in time.

The mass spectrum of ephedrine has a large peak at 77 amu, while the mass spectrum of heptanesulfonic acid has no peak at 77 amu. Heptanesulfonic acid has a large peak at 70 amu while ephedrine lacks this peak. Using this information we ask the computer to draw a limited mass plot for 77 amu and 70 amu as depicted in Figures #4 and #5. The computer has now separated the total ion chromatogram for the ephedrine - ephedrine complex mixture into separate ion chromatograms; one depicting ephedrine and the other the complex. The mass spectra obtained from these limited mass plots were indeed those of ephedrine and ephedrine heptanesulfonate complex. A mass spectrum of ephedrine heptanesulfonate complex contains peaks from the ephedrine as well as the heptanesulfonic

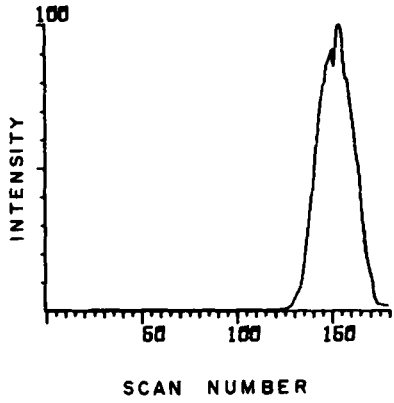

FIGURE 4

Limited Mass Plot for mass 77 of ephedrine extracted at pH 9.

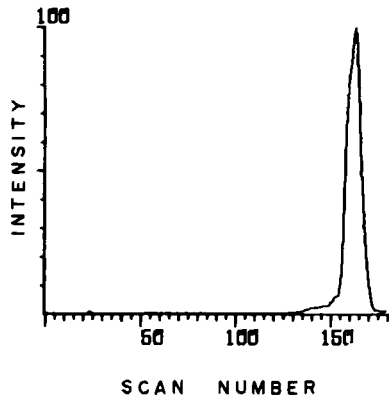

FIGURE 5

Limited Mass Plot for mass 70 of ephedrine complex extracted at pH 9.

acid mass spectrum. At pH 11 the total ion chromatogram and the limited mass plot for 77 were almost identical. It was not possible to obtain a limited mass plot for 70; therefore no complex was present. Only at pH 10 and above were IR's obtained on the extracts free of interferences. This again is consistent with complex being extracted at lower pH values.

The same experiment as above was repeated for methamphetamine hydrochloride at pH 9, 10, and 11 with similar results. The amount of methamphetamine (calculated as the hydrochloride salt) recovered was 7.4, 8.2, and 7.4 mg. respectively. The MS with data system showed appreciable complex at pH 9, a small amount of complex at pH 10, and a trace of complex at pH 11. The IR of the hydrochloride salt of methamphetamine extracted from a pH 9 solution was distorted. At pH 10 the resultant IR had a small distortion while at pH 11 the IR was undistorted.

Unexpected results were obtained when repeated collections from ten milligram ephedrine hydrochloride injections were extracted at different pH's from a mobile phase containing 0.04M methanesulfonic acid. As Table 2 indicates, the amount of ephedrine recovered (calculated as the hydrochloride salt) is zero at pH 3.5 and gradually increases to a maximum at pH's 11 and 12. This data indicates that no complex is being extracted. This is supported by the MS and IR data. It is not known at this time if, in general, methanesulfonate complexes are not extracted into chloroform. However, for maximum yield, the pH chosen for extraction into chloroform should be at least 1.5 units above the pKa value of the base.

TABLE 2

Calculated yields from 10 mg. injections on a Magnum 9 column of ephedrine hydrochloride extracted at various pH values from a mobile phase with 20% methanol, 79% water, 1% acetic acid containing 0.04M methanesulfonic acid at 3.5. Chromatography conditions for ephedrine assay: See Table 1.

pH	Milligram Ephedrine (calc. as HCl)
direct	--
7	1.5
8	3.1
9	4.6
10	6.3
11	8.7
12	8.7

Neutral drugs or those which exist as the free acid at pH 3.5 could be extracted directly into chloroform since no complex would be formed.

APPLICATIONS

The method was applied to various laboratory submissions for which a few examples will be illustrated.

A methanolic solution of a powder allegedly containing LSD was injected onto an analytical column and found to contain two large peaks consistent with LSD and iso-LSD, see Figure #6. The same solution was injected onto a semi-preparative column containing the same mobile phase as above except the counter ion concentration was 0.04M. As Figure #7 illustrates a chromatogram with similar resolution to that of Figure #6 was obtained. After evaporating a portion of the above solution to dryness on a steam bath under air, the residue in the mobile phase was injected onto the semi-preparative column and two fractions were collected. The fractions were extracted with chloroform from an adjusted mobile phase of pH 10. An IR on the resulting residue of the first collected fraction matched standard LSD base while the extract from the next fraction gave an IR matching iso-LSD. Both IR's were run as a film on a KBR window. The MS of the extracted fractions matched LSD. Since the semi-preparative injection represented approximately 21 mg. of sample, the k' values and resolution, as expected, decreased as illustrated in Figure #8.

Figures #9 and #10 show that the analytical profile in methanol of a methamphetamine street sample on a u-Bondapak C-18 and a Magnum 9 ODS column gave similar resolution. A dry chloroform extract of

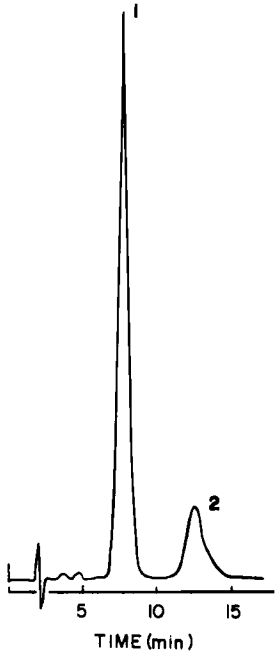

FIGURE 6

Analytical chromatogram of a clandestine LSD sample: (1) LSD; (2) iso-LSD. Conditions: Column u-Bondapak C-18; fixed wavelength detector sensitivity 0.04 a.u.f.s.; mobile phase, water, methanol, acetic acid (59, 40, 1) with 0.005M methanesulfonic acid adjusted to pH 3.5 as described in text; flow rate, 2.0 ml/min.

the powder was employed for the semi-preparative run, which is illustrated in Figure #11. Two collected fractions were adjusted to pH 11.5 and extracted with chloroform. A few drops of concentrated hydrochloric acid were added to the chloroform prior to evaporation on the steam bath. IR's of the collected fractions matched standard ephedrine hydrochloride and methamphetamine hydrochloride respectively.

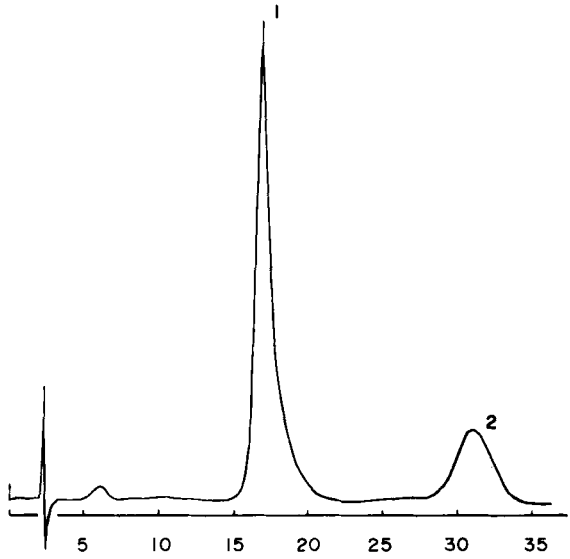

FIGURE 7

Analytical chromatogram of a clandestine LSD sample: (1) LSD; (2) iso-LSD. Conditions: Column, Magnum 9 ODS; fixed wavelength detector sensitivity 0.01 a.u.f.s.; mobile phase, water, methanol, acetic acid (59, 40, 1) with 0.04M methanesulfonic acid adjusted to pH 3.5 as described in text; flow rate, 5.0 ml/min.

A third example is a multi-barbiturate capsule which contained 100 mg. each of sodium amobarbital and sodium secobarbital. Again the analytical profiles of a methanolic solution of the capsule material on both the analytical and semi-preparative columns show good agreement as to resolution, see Figures #12 and #13. The contents of one capsule was extracted from 0.1N H_2SO_4 using chloroform prior to injection on the Magnum 9 ODS column. In this instance the same mobile phase as in the u-Bondapak C-18 column was used since acetic

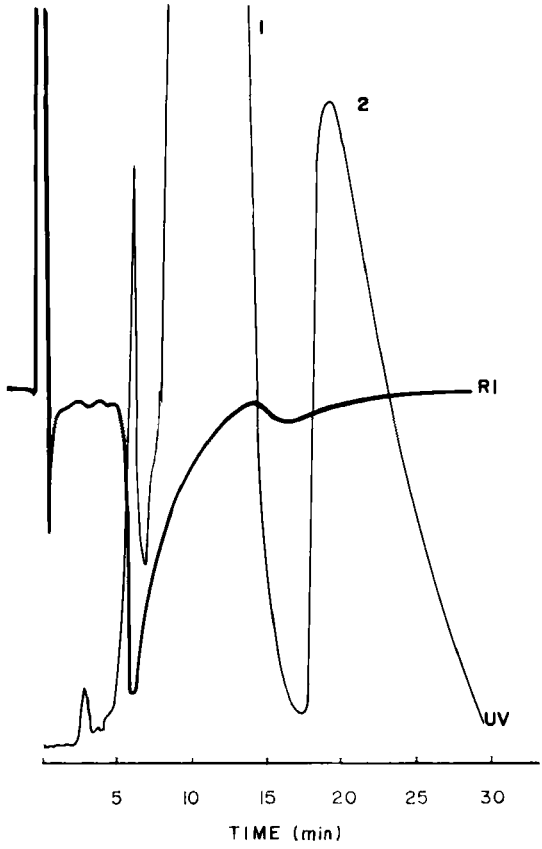

FIGURE 8

Semi-preparative chromatogram of a clandestine LSD sample: (1) LSD; (2) iso-LSD. Conditions: Column, Magnum 9 ODS; fixed wavelength detector sensitivity 2.0 a.u.f.s., RI 16X; mobile phase and flow rate, see Figure 7.

acid drugs such as amobarbital and secobarbital are not affected by counter ion[1]. This is illustrated in Figure #14 where the k' values of amobarbital and secobarbital for the milligram amounts of

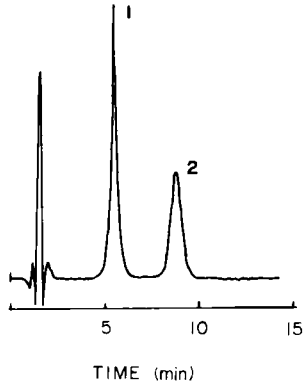

FIGURE 9

Analytical chromatogram of a methamphetamine street sample whose peaks represent: (1) ephedrine; (2) methamphetamine. Conditions: Column u-Bondapak C-18; fixed wavelength detector sensitivity 0.01 a.u.f.s., mobile phase, water, methanol, acetic acid (79, 20, 1) with 0.005M pentanesulfonic acid adjusted to pH 3.5 as described in text; flow rate, 2.0 ml/min.

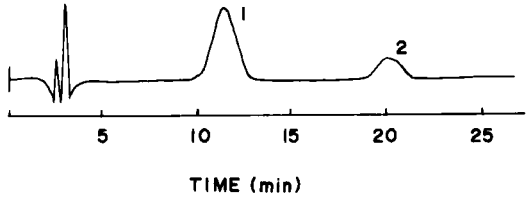

FIGURE 10

Analytical chromatogram of a methamphetamine street sample whose peaks represent: (1) ephedrine; (2) methamphetamine. Conditions: Column, Magnum 9 ODS; fixed wavelength detector sensitivity 0.005 a.u.f.s.; mobile phase, water, methanol, acetic acid (79, 20, 1) with 0.04M pentanesulfonic acid adjusted to pH 3.5 as described in text; flow rate, 5.0 ml/min.

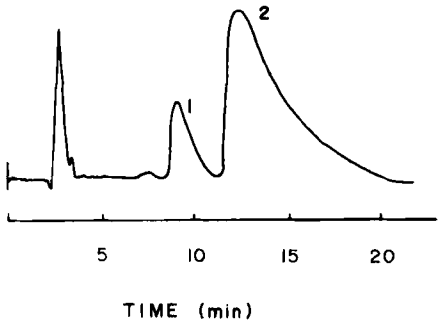

FIGURE 11

Semi-preparative chromatogram of a methamphetamine street sample whose peaks represent: (1) ephedrine; (2) methamphetamine. Conditions: Column, Magnum 9 ODS; fixed wavelength detector sensitivity 2.0 a.u.f.s.; mobile phase and flow rate, see Figure 10.

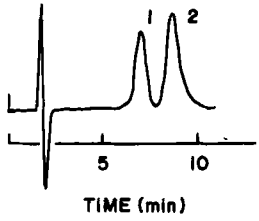

FIGURE 12

Analytical chromatogram of a multi-barbiturate capsule: (1) amobarbital; (2) secobarbital. Conditions: Same as Figure 6 except detector sensitivity 0.01 a.u.f.s.

material injected do not change relative to an analytical injection. The two collected fractions were extracted with chloroform and gave IR's that match standard amobarbital and secobarbital.

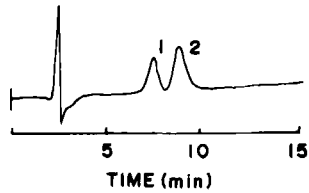

FIGURE 13

Analytical chromatogram of a multi-barbiturate capsule: (1) amobarbital; (2) secobarbital. Conditions: Column, Magnum 9 ODS; fixed wavelength detector sensitivity 0.01 a.u.f.s.; mobile phase, see Figure 6; flow rate, 5 ml/min.

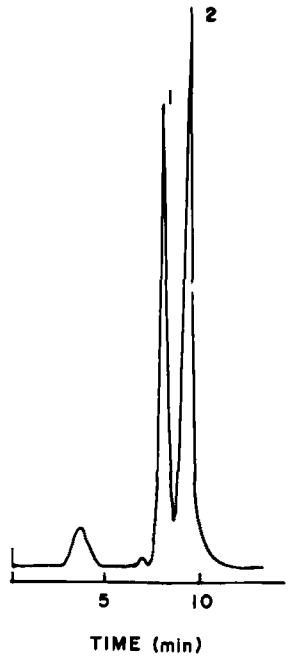

FIGURE 14

Chromatogram of an acid extract of multi-barbiturate capsule. (1) amobarbital; (2) secobarbital. Conditions: Column, Magnum 9 ODS; fixed wavelength detector sensitivity 2.0 a.u.f.s.; mobile phase, see Figure 6; flow rate, 5 ml/min.

Other samples in which this method was successfully applied included LSD in dyed blotter spots, lysergic acid amide and iso-lysergic acid amide in morning glory seeds, amphetamine in "minnibennie" tablets, cocaine in a street sample and clandestinely manufactured methaqualone tablets.(7)

One exhibit consisted of a small quantity of colored microdot tablets containing 7 mcg. each of LSD. A MS of LSD was obtained from a concentrated sample injected onto a u-Bondapak C-18 column using the same mobile phase employed for analytical injections.

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