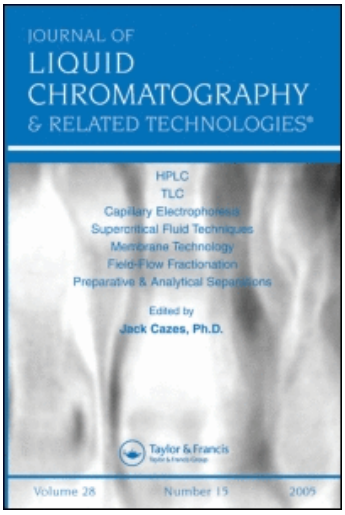

Journal of Liquid Chromatography & Related Technologies

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

Improved Isocratic Mobile Phases for the Reverse Phase Ion-Pair Chromatographic Analysis of Drugs of Forensic Interest

Ira S. Lurie[a]

[a] Drug Enforcement Administration Northeast Regional Laboratory, New York, New York

To cite this Article Lurie, Ira S.(1981) 'Improved Isocratic Mobile Phases for the Reverse Phase Ion-Pair Chromatographic Analysis of Drugs of Forensic Interest', Journal of Liquid Chromatography & Related Technologies, 4: 3, 399 — 408

To link to this Article: DOI: 10.1080/01483918108059944

URL: http://dx.doi.org/10.1080/01483918108059944

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(3), 399-408 (1981)

IMPROVED ISOCRATIC MOBILE PHASES FOR THE REVERSE PHASE ION-PAIR CHROMATOGRAPHIC ANALYSIS OF DRUGS OF FORENSIC INTEREST

Ira S. Lurie

Drug Enforcement Administration
Northeast Regional Laboratory
555 West 57th Street
New York, New York 10019

ABSTRACT

Most drug exhibits of forensic interest can be analyzed by reverse phase ion-pair chromatography using a Microbondapak-C-18 column with two isocratic mobile phases. These systems employing methanesulfonic acid represent a great improvement in resolution and speed over the author's original methodology.

INTRODUCTION

High Performance Liquid Chromatography has proven to be an excellent technique for the analysis of drugs of forensic interest[1-3]. It is particularly suited for drugs which are difficult to analyze by gas chromatography, such as nonvolatile, polar and thermally degradeable compounds. Examples of such drugs are phenethylamines, ergot alkaloids and certain opium alkaloids. In addition, since the technique is non-destructive, compounds can be isolated for identification by spectrometric techniques. Reverse phase ion-pair chromatography has been applied for the separation of a wide range of drugs of forensic interest[4]. This technique employed a buffered aqueous-organic

mobile phase containing a heptanesulfonate anion which was available to form an ion-pair complex with the cationic salt of a basic drug. In this system consisting of 40% methanol, 59% water, 1% acetic acid and 0.005M heptanesulfonic acid at a pH of approximately 3.5, acidic drugs could be analyzed by ion suppression. Thus, this system offered great versatility since it allowed the simultaneous analysis of acidic and basic drugs. Certain drawbacks existed in terms of resolution and speed. In order to optimize these separations of forensic interest studies were carried out to ascertain the effect of column, stationary phase, water-methanol ratio, counter ion size, counter ion concentration and basicity of drug chromatographed on a reverse phase ion pair chromatographic separation for approximately 50 drugs of forensic interest[5, 6]. These studies demonstrate that most drug exhibits could be analyzed using only two isocratic systems.

EXPERIMENTAL

The liquid chromatograph consisted of the following components: Model 6000A pump (Waters Associates, Milford, MA); Model U6K Injector or Model WISP 710A Auto Injector (Waters); prepacked 3.9 mm x 30 cm. stainless steel column, Microbondapak C-18 (Waters); Model 770 variable UV detector at 254 nm. (Schoeffel Instruments, Westwood, NJ) or Model 440 fixed UV detector at 254 nm (Waters); Systems 1VB intergrator (Spectra Physics, Santa Clara, CA).

Materials

The following chemicals were employed: methanesulfonic acid sodium salt (Eastman Chemicals, Rochester, NY); methanol, distilled

in glass (Burdick and Jackson, Muskegon, MI); distilled water and other chemicals were reagent grade. Authentic drug standard of USP/NF quality were employed.

Procedures

The mobile phases were prepared by dissolving methanesulfonic acid in a solution consisting of glacial acetic acid, methanol and distilled water. After filtering and degassing the solution through a Millipore 0.50 micron filter (Millipore Corporation, Bedford, MA), the pH was adjusted to 3.5 with 2N NaOH. All standards were dissolved in methanol.

RESULTS AND DISCUSSION

Most drug exhibits could be analyzed using only two mobile phases with a Microbondapak C-18 column. One such mobile phase consists of 40% methanol, 59% water, 1% acetic acid and 0.02M methanesulfonic acid at pH 3.5. This mobile phase is recommended for drug samples containing barbiturates, local anesthetics, LSD and related compounds, PCP and methaqualone. A chromatogram of standard barbiturates is shown in Figure 1. In a previous paper it was shown that when using reverse phase ion pairing chromatography the retention time of bases can vary with solute concentration, especially at higher concentrations[4]. A 0.02M methanesulfonic acid concentration was used instead of 0.005M because the variation in retention time of bases with sample was significantly diminished in the usual working sample concentration range which is < 1.0 mg/ml. The relative and actual retention times of various drugs analyzed on this mobile phase did not vary with counter

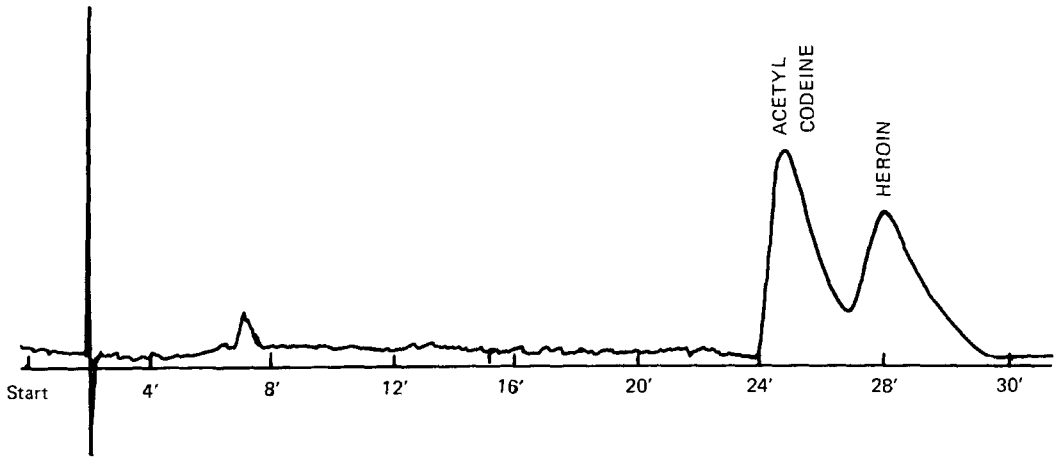

Figure 1 - Chromatogram of a standard mixture of barbiturates. Conditions - column Microbondapak-C18; variable wavelength detector sensitivity 0.02 a.u.f.s.; mobile phase - water, methanol, acetic acid (59, 40, 1) as described in text; flow rate - 2.0 ml/min.

ion concentrations. The retention times of local anesthetics except for benzocaine, which is not affected by counter ion, are significantly reduced by the changing of the counter ion from heptanesulfonate to methanesulfonate. A mobile phase containing a heptanesulfonate counter ion, as explained earlier, was used in an earlier paper. This mobile phase of 40% methanol, 59% water, 1% acetic acid with 0.005M heptanesulfonic acid at pH 3.5 was found to give excessive retention times for cocaine samples. By using a methanesulfonate counter ion, good resolution was still maintained and the retention of cocaine and tetracaine wa reduced by at least a factor of four. Similarly by reducing the counter ion from heptanesulfonate to methanesulfonate the retention times of PCP LSD and its related compounds are significantly reduced while excellent resolution is still obtained. The second mobile phase consists of 20% methanol, 79% water, 1% acetic acid and 0.02M methanesulfonic acid at a pH of 3.5. This mobile phase is optimum for phenethylamines. A 0.02M methanesulfonic acid concentration was again utilized for reasons covered earlier. As before the actual and relative retention times did not vary significantly with counter ion concentration. A major drawback of using the 40% methanol, 59% water, 1% acetic acid with 0.005M heptanesulfonic acid at a pH of 3.5 for the analysis of phenethylamines was the inadequate resolution between amphetamine and methamphetamine. It was shown earlier[6] that for a given column and counter ion the selectivity factor between amphetamine and methamphetamine increased significantly in going from 40% to 20% methanol. The selectivity between these two compounds are not adversely affected if while keeping the water-methanol ratio constant the counter ion size is changed. This effect is

true in general for the bases studied. Although going from 40% to 20% methanol would increase retention, this effect is offset by lowering the size of the counter ion. Phenylpropanolamine, ephedrine, amphetamine, methamphetamine, phentermine and caffeine and baseline resolved in approximately ten minutes (Figure 2). The retention times of these compounds are comparable with those found using the 40% methanol 0.005M heptanesulfonic acid solution. In a previous paper by Lurie, it was shown that heroin and acetylcodeine would co-elute using the latter mobile phases[4]. We have shown in a previous paper that a given counter ion for the C-18 column, the selectivity for heroin and acetylcodeine will increase with the amount of water in the mobile phase[6]. By changing the percent methanol from 40 to 20 and counter ion from heptanesulfonate acid to methanesulfonate, heroin and acetylcodeine were separated with a resolution of approximately one (Figure 3). It appears that a good approach for improving the resolution for closely related compounds with reverse phase ion pair chromatography is to increase the amount of water and lower the size of the counter ion in the mobile phase if possible.

The 20% and 40% methanol-methanesulfonic acid solutions with the Microbondapak C-18 column have been in routine use in our laboratory for almost two years with good success. Various assays for methamphetamine, cocaine, PCP and LSD were found to be in good agreement when compared with alternative techniques such as ultraviolet and fluorescence spectroscopy and gas chromatography. Linearity studies were performed on various drugs. Using 10 lamda injections, phenobarbital was found to be linear at least between 0.125 mg. and 4.0 mg. per ml. meth-

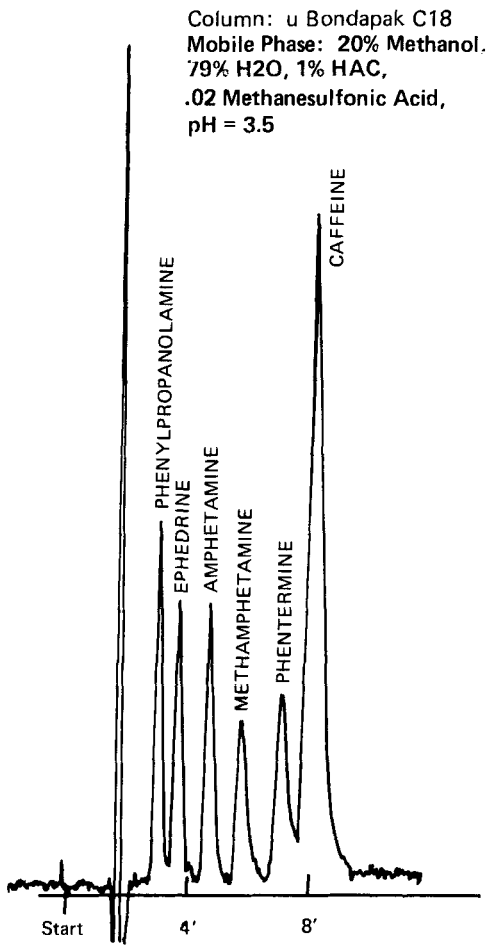

Figure 2 - Chromatogram of synthetic mixture of phenethylamines and related compounds. Conditions - same as Figure 1 except mobile phase is water, methanol, acetic acid (79, 20, 1) with 0.02M methanesulfonic acid adjusted to pH 3.5 as described in text.

anol. No change in retention time was noted. Also using 10 lamda injections, cocaine was linear between at least 0.125 and 4.0 mg. per ml. methanol. At a concentration greater than 1.0 mg. per ml. a significant decrease in retention with concentration was noted. PCP, using

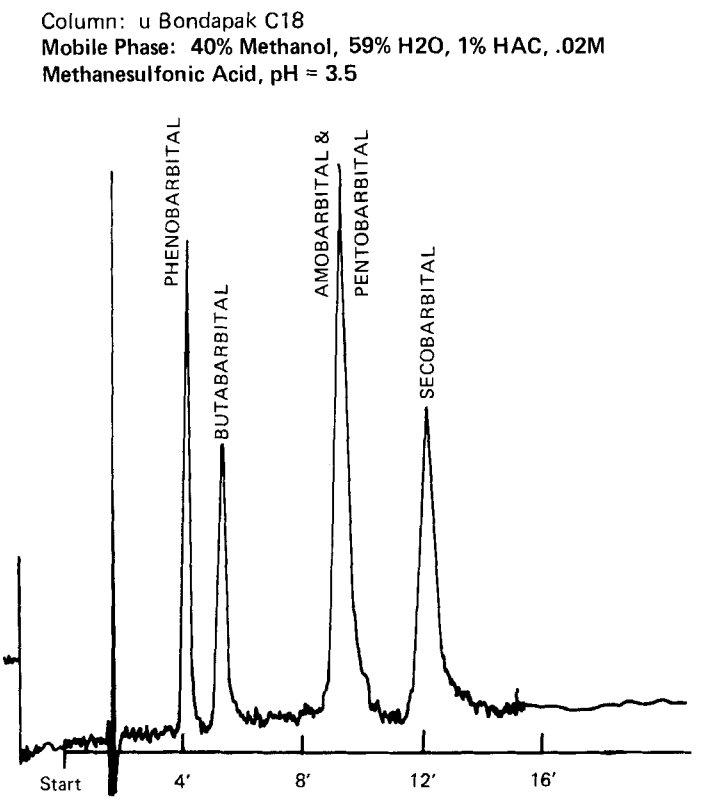

Figure 3 - Chromatogram of standard heroin and acetyl-codeine. Conditions - same as Figure 2.

20 lamda injections, is linear between at least 0.25 mg. and 4.0 mg. per ml. methanol. PCP exhibited an appreciable variation in retention time with concentration. Using 20 lamda injections, LSD is linear between at least 10 micrograms and 153 micrograms per ml. The retention time did not vary significantly with concentration. For 10 lamda injections of methaqualone linearity between at least 0.125 mg. and 4.0 mg. per ml. was observed. Only at the highest concentration range did the retention time vary significantly with concentration. Metham-

phetamine using 10 lamda injections was linear from at least 0.25 mg. to 4.0 mg. per ml. Above a concentration of 1 mg. per ml. the retention time varied significantly with concentration of the sample. Samples were prepared as previously shown[4]. For drugs slightly soluble in methanol, eg, amphetamine sulfate, starting mobile phase is recommended to dissolve the sample.

Most of the drugs that could be analyzed on the C-18 column using the 20% and 40% methanol, methanesulfonic acid solutions could be analyzed on the alkylphenyl column with these same mobile phases as recent studies indicate[5, 6]. In the same investigation the Microbondapak-CN column was found to be lacking selectivity for many of the drugs chromatographed, especially for drugs that ion pair. Therefore, this column is not recommended for the general analysis of drugs of abuse. Retention data for the various recommended mobile phases is presented elsewhere[6].

CONCLUSION

This paper demonstrates that most drugs commonly encountered in a forensic laboratory could be analyzed using two isocratic systems with a Microbondapak C-18 column or a Microbondapak Alkyl Phenyl column. The Microbondapak-CN column is not generally recommended.

The data presented in recent studies could serve as a guide for which column and mobile phase combinations to use in the separation of complex drug mixtures which are not separated by the recommended mobile phases.

REFERENCES

1. Jane, I. J., Chromatogr. 111, 227-232, 1975.

2. Wittwer, J. D., J. Forensic Sci. 18, 138-142, 1973.

3. Wittwer, J. D. and Kluckholn, J. H., J. Chromatogr. Sci. 11, 1-3, 1973.

4. Lurie, I., J. Ass. Off. Anal. Chem., 60, 1035-1040, 1977.

5. Lurie, I., Demchuk, S., J. Liq. Chromatogr., 1981, in press.

6. Lurie, I., Demchuk, S., J. Liq. Chromatogr., 1981, in press.