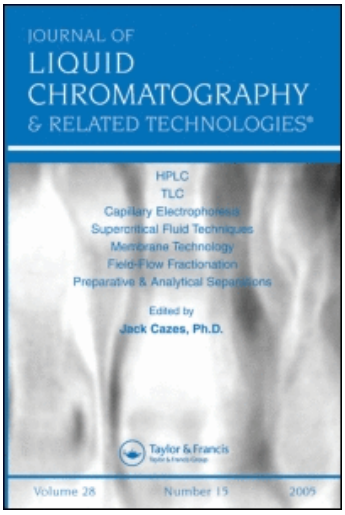

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High-Pressure Liquid Chromatographic Separation of Diphenhydramine and Some of its Metabolites: Effects of Eluent Salt Concentration on Chromatographic Characteristics

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HIGH-PRESSURE LIQUID CHROMATOGRAPHIC SEPARATION OF DIPHENHYDRAMINE AND SOME OF ITS METABOLITES : EFFECTS OF ELUENT SALT CONCENTRATION ON CHROMATOGRAPHIC CHARACTERISTICS

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ABSTRACT

With appropriate variation of the pH, alkali halogenide concentration and cation and/or anion species in the mobile phase (mixtures of water and methanol), it was possible to resolve diphenhydramine, N-desmethyldiphenhydramine, N,N-didesmethyldiphenhydramine, benzhydrol, benzophenone and 2-methyldiphenylmethoxy acetic acid on a reversed-phase column. The retention volumes of the three amines decreased by increasing the following parameters : salt concentration, methanol concentration and cation and/or anion size. The retention volumes of the other compounds were not influenced. The good separation and the simplicity make this method attractive for use in metabolism studies.

INTRODUCTION

An analytical procedure, commonly used for the determination of diphenhydramine and other antihistamines, involves solvent extraction followed by gas liquid chromatography (1-4). For metabolism studies, however, this technique is less suitable. Some diphenhydramine metabolites, e.g. the N-oxide (5), are rather thermolabile, and others such as diphenylmethoxy acetic acid and the conjugation products are not volatile enough.

In the present study high-pressure liquid chromatography (HPLC) was tried in attempting to devise a procedure by which the above objections are met.

In the literature dealing with HPLC a large variety of liquid chromatographic modes has been reported, even if one confines to compounds which are more or less structurally related to diphenhydramines. Knox and Jurand (6) described the separation of catecholamines and their metabolites using adsorption chromatography, ion-pair chromatography and soap chromatography. In the first case they noticed the problem of irreversible adsorption. This might soon impair the column. Ion-pair chromatography usually requires an organic solvent as mobile phase. This necessitates extractions of the compounds to be analyzed from body fluids, bringing about the risk of loss or rearrangement of metabolites during analysis. When the mobile phase is of the aqueous type, the body fluid can be injected directly onto the column (after deproteinization). Therefore, in general, all modes requiring an organic mobile phase are less suitable for drug metabolism studies. Soap chromatography seemed to be very promising, but in the case of low detergent concentrations it has the disadvantage that elution order will completely be changed on varying the detergent concentration. Especially in studies, where the compounds to be separated are unknown, this can cause serious problems.
Ion exchange chromatography can be considered as well. Twitchett et al. (7) described the use of this mode for drug analysis. However, simultaneous separation of metabolites can be very complex, as neutral components will elute with the solvent front, and the elution pattern of weak acidic and basic ones can markedly be influenced by changes in pH and ionic strength. In the separation on an octadecylsilane stationary phase several mechanisms may be involved. In a study on the effect of eluent salt concentration on the chromatographic properties of basic compounds using an octadecylsilane stationary phase (with 66% underivatized silanol groups) and aqueous methanol mobile phases Sugden et al. (8) observed that next to liquid-liquid partitioning salting-out effects, ion-pair formation and ion exchange - although relatively high electrolyte concentrations were used - played a role in the retention. Twitchett and Moffat (9) evaluated the use of an

octadecylsilane stationary phase (a μBondapak C_{18} column) for analysis of drugs. For basic drugs they found a poor column efficiency. However, it should be noted that these authors used eluents containing 25mM phosphate buffer. Assuming that only 1% of the silanol groups are underivatized[+], it is most likely that at this high ionic strength the ion-exchange characteristics have completely been suppressed.

The purpose of the present work was to investigate the usefulness of the ion-exchange properties of octadecylsilane for the separation of diphenhydramine and the following (possible) metabolites : N-desmethyldiphenhydramine, N,N-didesmethyldiphenhydramine, benzhydrol (diphenylmethanol) and benzophenone*, using aqueous methanol mobile phases and electrolyte concentrations lower than commonly used in reversed-phase chromatography.

EXPERIMENTAL

Apparatus

A Waters Assoc. (Milford, Mass., U.S.A.) high-performance liquid chromatograph model ALC/GPC 204 was used throughout the study. The system was equipped with a fixed-wavelength 254-nm detector and a 12.5-μl flow cell. The flow-rate of the solvent mixture was 1.2ml/min. Chromatograms were recorded on a Kipp model BD8 single-pen recorder with a 1 - 10mV span.

Column

The chromatograph was fitted with a 30 cm long, 0.7 mm I.D., 4 mm I.D. reversed-phase μBondapak C_{18} column.

+ Claim of the manufacturer.

* 2-Methyldiphenylmethoxy acetic acid was added to this series. Unsubstituted diphenylmethoxy acetic acid was not available.

Solvents and chemicals

The mobile phase consisted of a mixture of methanol and distilled water (78/22 or 85/15 (V/V)), containing NaCl, NaBr, NaF, KCl or LiCl in several concentrations (0 - 3.42 mM). Methanol and the inorganic salts were of analytical grade. Diphenhydramine, N-desmethyldiphenhydramine, N,N-didesmethyldiphenhydramine, benzydrol, benzophenone and 2-methyldiphenylmethoxy acetic acid came from the laboratory stock.

Sample preparation

Injection samples were prepared by dissolving the parent compound and the metabolites in methanol-water mixtures. Sample solvents and eluents had always the same composition.

RESULTS AND DISCUSSION

Fig. 1. shows a successful and reproducible* separation of diphenhydramine, some of its metabolites, benzophenone (a possible product of O-dealkylation next to benzhydrol) and 2-methyldiphenylmethoxy acetic acid - a metabolite of orphenadrine, a derivative of diphenhydramine -. This was produced by 78% methanol in 1.71 mM aqueous sodium chloride adjusted to pH 7.0. Since it was supposed that ion exchange was involved in this separation, special attention has been paid to the effects of pH, ionic species and salt concentration of the mobile phase on chromatographic behavior.

The retention times of the three amines were clearly affected by the pH. When using an unbuffered 78/22 methanol/water eluent adjusted to pH = 3, the amines eluted with the front. At pH = 7

* All experimental results combined, the standard deviation in the retention time lies within the range 0.55 and 0.83 per cent of the mean. The separation was performed twenty times.

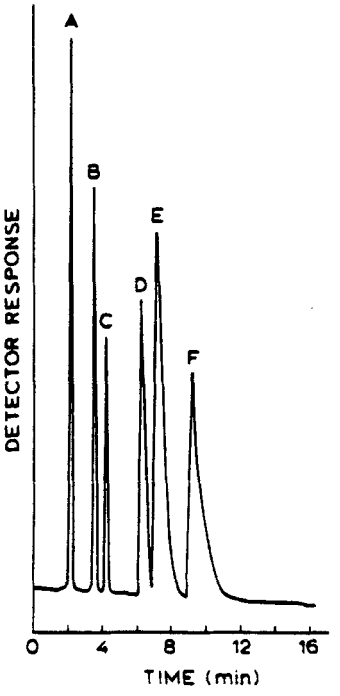

Fig. 1 -High-pressure liquid chromatogram showing (A) 2-methyldiphenylmethoxy acetic acid, t_R = 3'22"; (B) benzhydrol, t_R = 3'39"; (C) benzophenone, t_R = 4'22"; (D) N,N-didesmethyldiphenhydramine, t_R = 6'27"; (E) N-desmethyldiphenhydramine, t_R = 7'21"; (F) diphenhydramine, t_R = 9'21". Solvent : methanol-water (78:22), pH = 7 and sodium chloride concentration = 1.71mM; flow-rate = 1.2 ml/min. The concentrations of the compounds were 50 µg/ml, 50 µg/ml, 1 µg/ml, 50 µg/ml, 25 µg/ml and 50 µg/ml, respectively; sample volume 20 µl.

(same methanol/water ratio) the amines did not elute at all. However, when at this pH sodium chloride was added the amines eluted.

The retention volumes of the amines decreased by increasing the following parameters : alkalihalogenide concentration (Fig. 2), cation and/or anion radius (Figs 3 and 4) (the first of which with

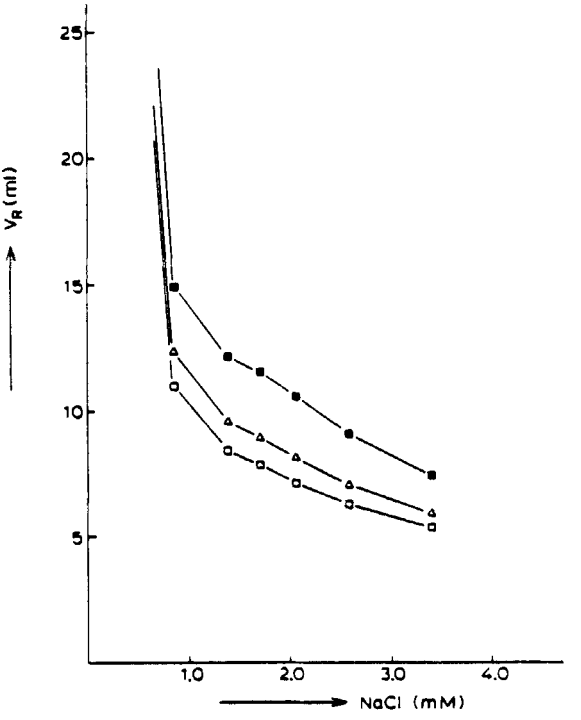

Fig. 2 -Effect of eluent sodium chloride concentration on retention volumes of diphenhydramine (■), N-desmethyldiphenhydramine (△) and N,N-didesmethyldiphenhydramine (□).

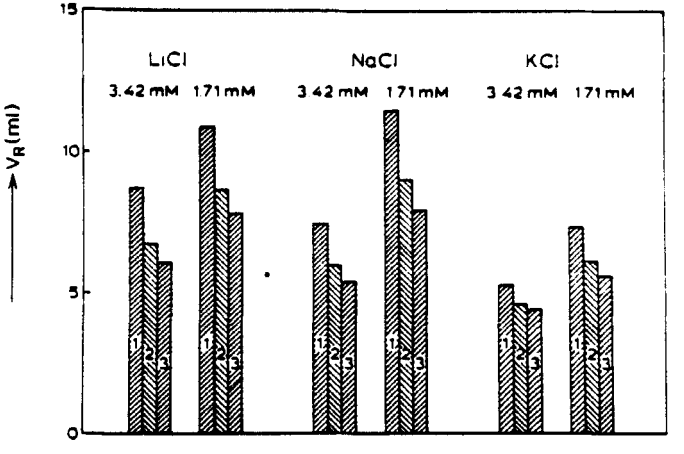

Fig. 3 -Effect of alkali cation species in eluent on retention volumes of diphenhydramine (1), N-desmethyldiphenhydramine (2) and N,N-didesmethyldiphenhydramine (3).

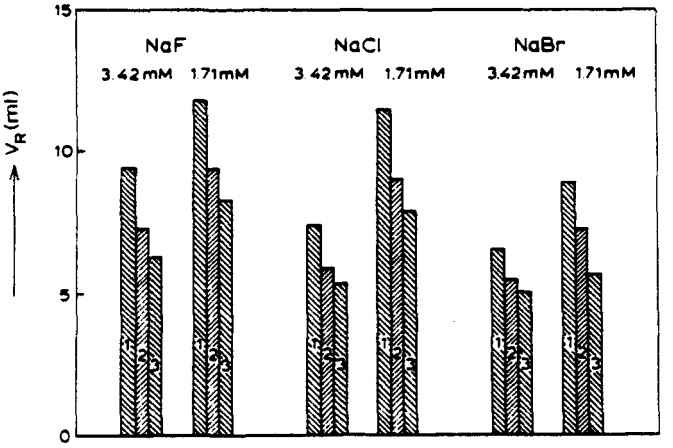

Fig. 4 -Effect of halogenide species in eluent on retention volumes of diphenhydramine (1), N-desmethyldiphenhydramine (2) and N,N-didesmethyldiphenhydramine (3).

the exception of 1.71 mM sodium chloride) and methanol concentration. The retention volumes of the other compounds reported in Fig. 4 were not influenced at all.

The above phenomena might be explained by assuming that the reversed-phase column has some cation-exchange properties, originating from underivatized silanol groups. First, the surface hydroxyl groups (Si - OH) will ionise above pH = 4 (10) (pK_a = 5.5 - 9) and create active sites for which the protonated amine and any cations in solution will compete. Secondly, the relationship between retention volumes and cation radius is in perfect agreement with the common order for cation exchange resins (11). Finally, the elution behavior of benzhydrol and benzophenone - both neutral compounds - as well as that of 2-methyldiphenylmethoxy acetic acid - at pH = 7 existing in the anion form - is also understandable on the basis of ion exchange.

The effect of the anion size upon retention volume indicates that some mechanism other than ion exchange is also involved

(Fig. 4). Ion-pair formation might be considered to be operative. The observed decrease of retention volume by increasing anion size is compatible with the observations of Evans and Matesich (12) who found that the association constants of some tetraalkylammonium halogenides in hydrogen-bonding solvents increased in the order $Cl^- < Br^- < I^-$. Additional evidence for an ion-pair mechanism was the finding that retention volumes of the amines decreased [illegible] by raising the methanol concentration from 78 to 85% (pH = 7, sodium chloride concentration = 1.71 mM). A similar phenomenon was observed by Sugden et al. (8).

The practical advantage of the phenomena described is obvious : the retention volumes of the diphenhydramines can be changed when necessary, e.g. when peaks of other metabolites (conjugation products, hydroxylated compounds etc.) give overlap, or when a shorter analysis time is required.

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