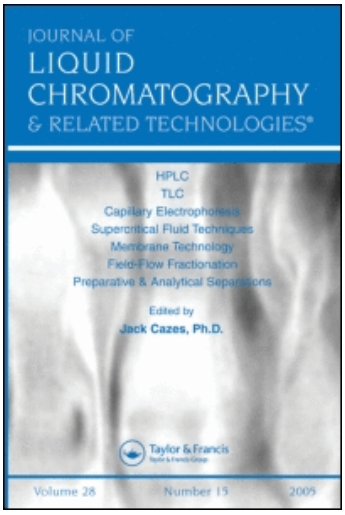

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Reversed Phase Ion-Pair Chromatographic Separation of Acetyl Choline and Choline

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REVERSED PHASE ION-PAIR CHROMATOGRAPHIC SEPARATION OF ACETYL CHOLINE AND CHOLINE

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ABSTRACT

A reversed phase High-Performance Liquid Chromatographic procedure for the separation of choline and acetyl choline is described. The method employs a mobile phase containing aromatic sulphonates as counter ions for ion-pair chromatography and for U.V. Visualisation of choline and acetyl choline at 254 nm. The method enables the separation of the two compounds in the 1-10 ug concentration range in six minutes. The separations are better achieved on a PRP-I resin column.

INTRODUCTION

Acetyl choline (Ach) and Choline (Ch) are physiologically important quarternary ammonium compounds - the former is an important neurotransmitter while the latter is a metabolic

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product of acetyl cholinesterase activity on Ach. The methods available for their determination include spectrophotometry[1,2] gas chromatography and pyrolysis gas chromatography[3].

D.N.Buchman et al[4] had reported a HPLC method for choline only by ion pairing the 3,5 dinitrobenzoate ester of choline (from plasma) with n-heptane sulphonic acid. An ion exchange method[5] is also available wherein radioactive labelled choline and acetyl choline have been separated on a cation exchange column and detected by scintillation counting. A reversed phase ion-pair chromatographic method for the determination of these using aromatic sulphonates as the counter ions in the mobile phase, which appears easier, is reported here.

Ion-pair chromatography in the reversed phase mode has been largely used for the separation of UV absorbing ionic species. UV absorbing counter ions have also been used for the UV visualisation and separation of non-UV absorbing ionic species. For example Bidlingmeyar[6] has reported the use of cetyl pyridinium bromide for the separation of alkyl sulphonates using UV detection.

EXPERIMENTAL

Waters Model ALC/GPC-244 Liquid Chromatograph was used for the study with the Model 440 UV detector set at 254 nm. The columns tried were: (1) Excalibar (25cm x 4.6cm i.d.) column packed with Spherisorb (5nm) ODS (Analabs): (2) u - Bondapak (c-18) and u Bondapak (CN); and (3) PRP-1 (Hamilton, Bonaduz) packed with styrene-divinyl benzene (10 um).

Chemicals and Solvents : Water used in the study had been triple distilled in an all glass distillation apparatus. The solvents acetonitrile and n-propanol were procured from E.Merck (India) and Ferak (Germany) and were used as such. Choline and acetyl choline were procured from E.Merck (Darmstadt) and Sigma

(USA) respectively. The mobile phase found suitable for the study was prepared from water, acetonitrile and n-propanol (90:7:3) and contained (a) potassium dihydrogen phosphate, E.Merck (0.45M), (b) p-amino benzene sulphonic acid (PABSA-BDH, ANALAR), $4x10^{-4}$M, and (c) cetyl trimethyl ammonium bromide (CTAB, Aldrich), $1x10^{-4}$M. The mobile phase pH was adjusted to 3.5 with sulphuric acid and filtered over a Millipore solvent filtration system fitted with a 0.25 u membrane filter (Schleicher & Schull, Dassel). Besides PABSA as the UV absorbing counter ion, p-toluene sulphonic acid was also tried. (See Fig.2 for Chromatographic conditions)

The sulphonates used in the study, especially PABSA, are sparingly soluble in water,but were rendered soluble in it by the presence of a quarternary ammonium detergent like CTAB. A stock solution of PABSA was prepared by dissolving the substance first in minimum volume of 0.5M detergent solution (2% v/v solution of CTAB in 20% aqueous methanol) and making up to the required volume in water.

Solutions of Ach and Ch (stock as well as working standards) were prepared in a 0.45M solution of KH_2PO_4 containing water,acetonitrile and n-propanol in the same proportions as the mobile phase. 1 ul volumes of these were injected.

Column Conditioning : The PRP-1 column used in the final study as well as the other columns used for preliminary work and mentioned earlier had to be thoroughly equilibriated with the mobile phase at ambient temperatures. The column was kept flushed with the mobile phase overnight at a flow rate of 0.2 ml/min and the flow rate was set at 2 ml/min for the actual analytical runs. The column was observed to attain equilibrium in 1 hr. The UV absorbance of the mobile phase due to the counter ion initially rose to a maximum and then gradually declined to a minimum (0.9 to 1 AUF). It could be offset at this stage with

TABLE

Separation of Choline and Acetyle choline on PRP-1 Column

Counter ion	Retention(min)		Lowest concentration detected in ug	
	Ch	Ach	Ch	Ach
1. p-Toluene Sulphonic Acid Sodium salt (PTSA)	3.45"	9.15"	5	2
2. p-Amino benzene Sulphonic Acid, Sodium salt(PABSA)	2.3"	6'	1	2

the ± offset potentiometric setting in the Model 440 UV Detector. Attainment of equilibrium was indicated by a steady baseline and observance of peaks with reproducible retention times for the test solutes. A certain degree of a slow upward baseline drift was found inevitable.

DISCUSSION

Preliminary trials involving naphthalene 2-sulphonic acid and p-toluene sulphonic acid as counter ions indicated that Ach and Ch could not be separated on the C18 and cyano bonded phases with varied solvent compositions. Separation was possible only on a PRP-1 resin column. So the method described here evolved around this column. The counter ions tested for separation on this column were : PTSA and PABSA. The retention times for Ch and Ach as also the lowest concentration detected

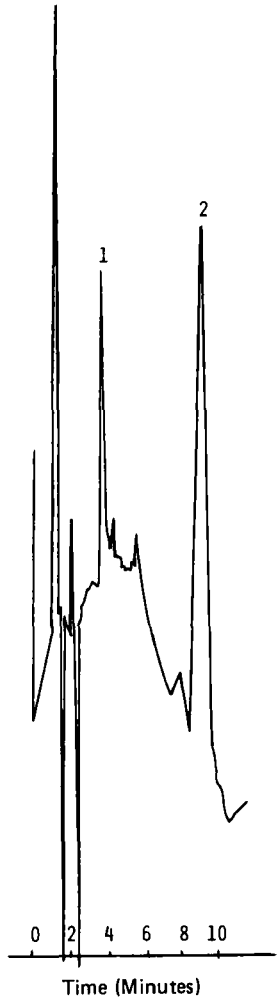

Figure 1. Separation of choline and acetyl choline by reversed phase ion-pair chromatography. Column: PRP-1 (Hamilton, Bonadu2). Mobile phase: 0.002M p-Toluene sulphonic acid in water; acetonitrile (90:10) containing potassium dihydrogen phosphate (0.75M) and cetyl trimethyl ammonium bromide ($1x10^{-4}$M) and adjusted to pH 6.5. Flow rate 1 ml/min and detection: UV at 254 nm and 0.01 AUF. Peak Identity: Ch, 6ug, peak No.1. Ach, 8 ug, peak No. 2.

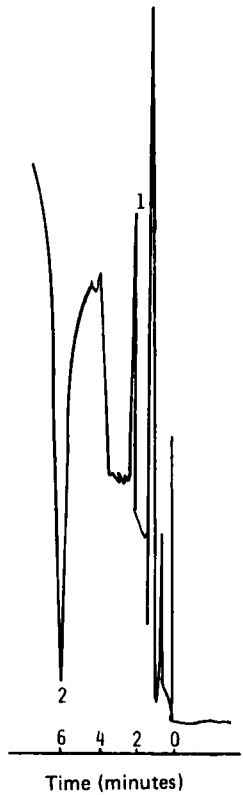

Figure 2. Separation of choline and acetyl choline by reversed phase ion-pair chromatography. Column: PRP-1 (Hamilton, Bondauz). Mobile phase: 0.4×10^{-4} M p-amino benzene sulphonic acid in water: acetonitrile: n-propanol (90:7:3) containing potassium dihydrogen phosphate (4.5×10^{-1}M) and cetyl trimethyl ammonium bromide (1×10^{-4}M) Peak Identity: Ch 6ug, peak No. 1. Ach, 8ug, peak No.2.

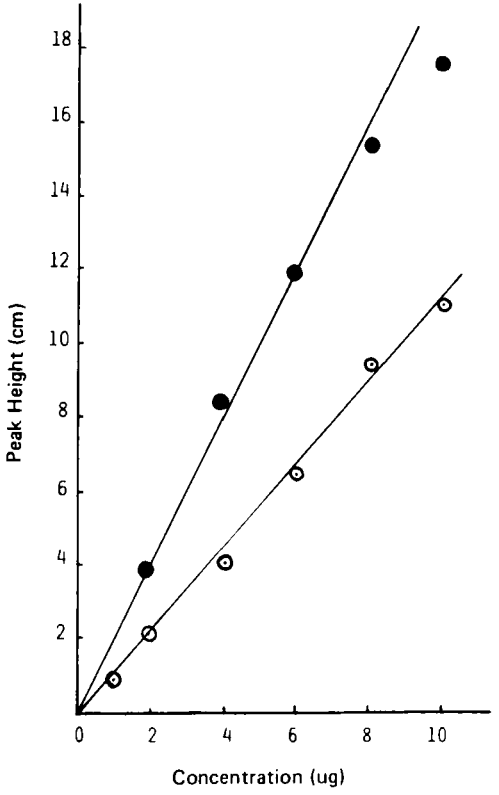

Figure 3. Calibration plots for choline and acetyl choline using PABSA as the counter ion. Conditions as in Figure 2. Ach (.), Ch (o).

(in micrograms) are mentioned in the table below. Chromatograms showing the separations of the two substances are shown in figures 1 and 2 alongwith the chromatographic conditions.

It can be seen from the above table that PABSA is a better counter ion, in view of the higher sensitivity attainable for both Ch and Ach. Acetyl choline emerges as a negative peak (Figure 2) with this counter ion in the mobile phase. Occurrence of negative peaks are not unusual in ion pair chromatography

with UV absorbing ions and have been reported by Bidlingmeyar[6]. They are also reported in indirect photometry on ion exchange columns employing UV absorbing anions or cations[7]. The calibration plots for Ch and Ach with the peak heights (cm) as a function of their concentration (ug) are linear and are shown in Figure 3. The plot for Ach is linear in the 2-8 ug range while that for Ch is linear in the 1-10 ug range.

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REFERENCES

1. Hestrin, S.J., J.Biol.Chem., 180, 249 (1949)
2. Mitchell, R., Clark, B.B., Proc.Soc.Exptl.Biol.Med., 81, 105 (1952)
3. Hasegawa, T., Kunihara, M., and Maruyama, Y., J.Chromatogr., 239, 335 (1982)
4. Buchmann, D.N., Fucek, F.R., and Domino, E.F., J.Chromatogr., 181, 329 (1982)
5. Stein, N.S., J.Chromatogr., 214, 148 (1981)
6. Bidlingmeyar, B.A., Warren, Jr, F.V., Anal.Chem., 54, 2351 (1982)
7. Larson, J.N., and Pfeiffer, C.D., Anal.Chem., 55, 393 (1983)