CHEMISTRY LETTERS, pp. 193-194, 1982.

ION-PAIR CHROMATOGRAPHIC DETERMINATION OF ORGANIC BASES USING COLOR REACTION OF TETRABROMOPHENOLPHTHALEIN ETHYL ESTER

Youko YOSHITOKU, Masataka MORIYASU*, and Yohei HASHIMOTO
Kobe Women's College of Pharmacy, Higashinada-ku, Kobe 658

By using TBPE as ion-pairing counter-ion whose absorption spectrum differs markedly from that of the resulting ion-pairs, ion pair high pressure liquid chromatographic determination of many organic bases was carried out sensitively without the absorptive background of TBPE added in the mobile phase.

Ion-pair chromatography has been used quite extensively since the first report by Eksborg and Schill[1]. In one of the modes of ion-pair chromatography, which is used in a normal-phase system, the ion-pairing counter-ion is physically coated on the surface of the stationary phase[1)-3)]. Although this system is applicable to as the color reaction, it has not been used extensively because of the short life time of column and the poor reproducibility due to gradual loss of the coated ion-pairing counter-ion by the mobile phase. The alternative mode involves separation in a reversed-phase system, being more popular and convenient[4)-7)]. This system is not applicable to as the color reaction which is the merit of ion pair extraction, because in this system ion-pairing counter-ion is added in the mobile phase and the absorption spectrum of ion-pairing counter-ion is usually similar to that of the resulting ion-pairs. However, if the absorption spectrum of the ion-pairing counter-ion differs markedly from that of the resulting ion-pairs, the resulting ion-pairs will be detected by means of the color reaction. we tried the ion-pair chromatography by tetrabromophenolphthalein ethyl ester(TBPE) as ion-pairing counter-ion whose color differs from that of the resulting ion-pairs.

TBPE which changes color near pH 4.5 - 5.5 is one of well known pH indicators. When the commercial TBPE potassium salt was dissolved in the polar solvents such as water, alcohol and acetonitril, these solutions showed blue color, indicating that TBPE reagent is present in an ionized form. On the other hand, when TBPE was dissolved in the halogenated hydrocarbon solvent, its solution showed yellow color, which shows that TBPE reagent is present in a molecular form. With many organic bases this reagent forms very stable ion-pairs extracted with organic solvents, these solutions being purple color. (absorption maximum : 550nm - 650nm, ε : 5×10^4 - 1×10^5) [8)-10)].

Eluent was obtained by the following alternative way.
(1) Dichloromethane was added to the aqueous solution of TBPE potassium salt, and the mixture was shaken vigorously. Most of TBPE was transferred into dichloromethane.
(2) The blue solution of TBPE potassium salt dissolved in alcohol or acetonitril was passed through the Amberlyst 15 (the cation exchange resion for nonaqueous

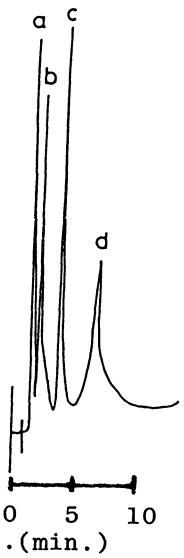
solvents), to obtain yellow TBPE eluent.

Columns packed with various stationary phases were tried in the separation of amines and alkaloids. Among these, the packings bonded $-NH_2$, -CN and -OH groups on the silica-gel gave the best results. The water content in the column influenced on the shape of peak or the retention factor. Since the peaks became sharp by the presence of a small amount of water in the column, the columns were preliminary treated by washing with an acetone-water mixture (100 : 5) containing a small amount of TBPE.

Chromatograms in Fig. 1 and Fig. 2 indicate separation of several organic bases as TBPE ion-pairs on Lichrosorb $-NH_2$ and Nucleosil-OH columns. Strychnine and brucine gave sharp peaks, whereas peak of *dl*-ephedrine tended to cause tailing. The peak of *dl*-ephedrine became sharp in Fig.2, but peak of *dl*-ephedrine still tended to be relatively broad. Thus many organic bases including substances which have weak uv-absorption such as (a) and (b) in Fig.2 can be detected sensitively by the present method.

Fig.1

Fig.2



Separation of amines and alkaloids

Fig.1
column: Lichrosorb $-NH_2$ (4mm x 15cm)
eluent: 5.0×10^{-4}mol dm^{-3}TBPE in
CH_2Cl_2 : $CH_3CH_2CH_2OH$ = 100 : 30
flow rate: 1.5 cm^3/min.
sample: a strychnine b brucine
c atropine d *dl*-ephedrine

Fig.2
column: Nucleosil-OH(4mm x 15cm)
eluent: 5.0×10^{-4}mol dm^{-3}TBPE in
CH_2Cl_2 : MeOH = 100 : 5
flow rate: 1.5 cm^3/min.
sample: a dibutylamine
b dipropylamine
c *dl*-ephedrine
d *dl*-norephedrine
detector: 572nm

The caliblation curve for brucine showed an approximately liner relationship from 10ng to 10μg. The detection limit of this detector was less than 10ng.

Further result will be published in a succesive report.

References

1) S.Eksborg and G.Schill, Anal. Chem., 45, 2092 (1973).
2) S.Eksborg, P.-O. Lagerstrom, R.Modin and G.Schill, J. Chromatogr., 83, 99 (1973).
3) B.Ulin, K.Gustavii and B.-A.Persson, J. Pharm. Pharmacol., 28, 672 (1976).
4) K.G.Wahlund, J. Chromatogr., 115, 411 (1975).
5) D.P.Wittmer, N.O.Nuessle and W.G.Haney, Anal. Chem., 47, 1422 (1975).
6) J.H.Knox and G.R.Laird, J.Chromatogr., 122, 17 (1976).
7) W.R.Melander, K.Kalghatgi and C.Horvath, J.Chromatogr., 201,201 (1980).
8) M.Tsubouchi, Nippon Kagaku Kaishi.,91, 1061 (1970).
9) M.Tsubouchi, Nippon Kagaku Kaishi.,91, 1190 (1970).
10) T.Sakai, I.Ogawa, M.Tsubouchi, and T.Kamata, Eisei Kagaku., 21, 199 (1975).

(Received November 9, 1981)