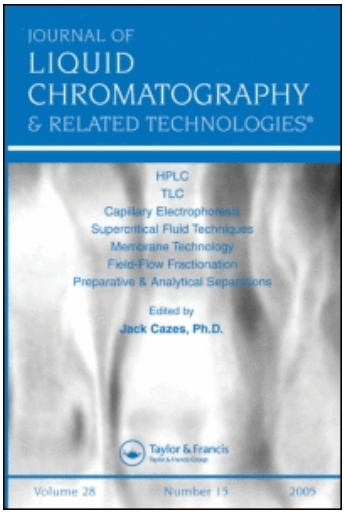

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DEUTERATED SOLVENTS AS MOBILE PHASE IN MICRO-HPLC

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ABSTRACT

The chromatographic performance of deuterated solvents, heavy water and deuteriobenzene, has been investigated in reversed phase and normal phase micro-HPLC. The performance of heavy water in separation was comparable or superior to that of light water in reversed phase mode. A slight improvement was also observed in catecholamines separation with heavy water. The performance of deuteriobenzene was observed as complicated in metal complexes separation, but a little difference was present. From those results, the combinations of micro HPLC and IR via flow-cell technique, proton-NMR, and ICP could be accomplished free from absorption or signals from mobile phase solvents.

INTRODUCTION

The selection of satisfactory separation conditions is still a major problem in liquid chromatography; two approaches are adapted. One is the control of column temperature, and a number of publications concerned therewith have recently appeared (1-6). The other is the control of mobile phase compositions. Several rules (7-9) have been presented for ascertaining a suitable mobile phase solvent for a specific chromatographic purpose.

In a case of higher selectivity and resolution being required, it becomes necessary to reconsider mobile phase systems. Novotny has demonstrated in his recent article (10) that micro-HPLC technique can

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provide the oppotunity to use "exotic solvents" as a mobile phase system because of an advantageous feature as smaller flow-rates as like on the order of microliters per minute.

Therefore the authors previously reported the chromatographic performance of deuterated solvents with micro-HPLC (11) such as CD_3OD and CD_3OD/D_2O in reversed phase mode, although some works on the use of deuterated solvents as mobile phase on conventional-HPLC have been already published (12-14). However, only very scant details of this subject were given there.

In this contribution, we will describe the more advanced discussion on the chromatographic performance of deuterated solvents in normal and reversed phase micro-HPLC.

EXPERIMENTAL

A micro-HPLC system consisted of a microfeeder MF-2(Azuma Electric, Co.Ltd., Tokyo, Japan) as a pump and a Jasco(Tokyo, Japan) Uvidec-100II UV spectrophotometer as a detector. The infrared detection was performed by JEOL(Tokyo, Japan) JIR-40X Fourier transform infrared spectrometer. The micro-HPLC columns used were as follows;

a) silica column: PTFE tubing (0.5mm i.d. x 15 cm length) packed with Jasco FineSIL-5(5 μm).

b) C 18 column : fused silica capillary (0.35 mm i.d. x 30 cm length) packed with Chemcosorb ODS/H(7 μm, Chemco, Osaka, Japan), PTFE tubing (0.5 mm i.d. x 12 cm length) packed with Jasco FineSIL C-18(10 μm) and PTFE tubing (0.5 mm i.d. x 15 cm length) packed with Jasco SC-01 (5 μm).

c) C-2 column : PTFE tubing (0.5 mm i.d. x 10 cm length) packed with Jasco FineSIL C-2(10 μm).

d) C-8 column : PTFE tubing (0.5 mm i.d. x 13 cm length) packed with Jasco FineSIL C-2(10 μm).

e) column for IR : PTFE tubing (0.65 mm i.d. x 44 cm length) packed with Jasco FineGEL SC-220(11.7 μm) attached with on-column flow-cell (15).

Those columns were prepared by the slurry technique (16).

Mobile phase solvents in reversed phase mode were HPLC grade methanol and acetonitrile, 99.75 % heavy water and purified water. Benzene and 99.73 % deuteriobenzene were used in normal phase separation. All of test substances except few metal complexes were commercially available products as received. Metal complexes of Co-,Cr-and Fe-diethyldithiocarbamates were synthesized in normal ways.

The temperature control of each column was performed by the thermostat of Komatsu DW-620 (Tokyo, Japan).

The standard samples were injected into columns as a few hundred ppm concentration of methanol or acetonitrile solution. Sodium nitrite for reversed phase mode (17,18) and FC-78(N-trifluoromethylperfluoro-morpholine, marketed from 3M Company, U.S.A.) (19) for normal phase mode were used as t_0-materials, respectively. The capacity factor, k', was calculated from the retention time of the eluate, t_R, according to the equation of $k' = (t_R - t_0)/t_0$.

All measurements were made in at least triplicate. The average reproducibility of each run was better than about 1 % relative.

RESULTS AND DISCUSSION

To examine the chromatographic performance of heavy water, separations of alkylbenzenes were performed on various columns. Table-1 shows the capacity factors of toluene, ethylbenzene and n-propylbenzene with

Table-1 Capacity factors of toluene, ethylbenzene and n-propylbenzene with light and heavy water-acetonitrile aqueous mobile phase systems.

acetonitrile concentration (%)	capacity factor, k' toluene H_2O	toluene D_2O	ethylbenzene D_2O	n-propylbenzene D_2O
30	12.0	14.8	28.4	64.5
40	4.90	6.32	10.3	18.5
50	2.58	3.10	4.42	6.64
60	1.45	1.64	2.12	2.90
70	0.66	0.76	0.94	1.24

Chromatographic conditions: column; 0.5 mm i.d. x 12 cm, C-18(FineSIL)
flow-rate; 20 µL/min
column temperature; 25 °C

various concentration of heavy water and light water in acetonitrile aqueous mobile phase. The slight increase of the capacity factors caused from the use of former as a mobile phase component is clearly observed in the results. The difference of the capacity factors of toluene between heavy water and light water is about 20 % relative. This is only small increment in retention, but the difference of both systems is apparently present. The results shown in Table-2 were obtained at the concentration of acetonitrile in mobile phases where toluene eluted at the same capacity factor. The retention behavior of alkylbenzenes in both systems is completely the same to the general reversed phase separation mechanism.

The retention factors of alkylbenzenes in acetonitrile and methanol aqueous mobile phase systems are tabulated in Table-3, in which (A) shows the result on C-2 column, (B) shows for C-8 and (C) shows for C-18, respectively. For all the systems, the capacity factors in using heavy

Table-2 Capacity factors of alkylbenzenes with light and heavy waters in acetonitrile aqueous mobile phase system.

compound	capacity factor, k'	
	CH_3CN/H_2O=80/20	CH_3CN/D_2O=82/18
toluene	0.68	0.68
ethylbenzene	0.96	0.95
propylbenzene	1.42	1.39
n-butylbenzene	2.09	2.04
n-amylbenzene	3.13	3.00
n-hexylbenzene	4.71	4.77

Chromatographic conditions: column; 0.35 mm i.d. x 30 cm, Chemcosorb.
flow-rate: 2 μL/min
column temperature; 25°C

water are larger than those with light water. No improvements with the former are observed in separation factors for acetonitrile mobile phase, where α is consistent with each other in ± 1.0 % relative, while small increases in α are found in methanol system. The separation performance depending on heavy water seems to relate with the kind of column packing materials, and their order is approximately depending on the length of bonded carbon chains.

It is clear from above results that the separation factors of alkylbenzenes are slightly improved with the use of heavy water instead of light water in methanol aqueous system and these effects are influenced from some characteristics of the stationary phase.

The second example to show the performance of heavy water is the separation of catecholamines. In the separation of catecholamines water is used as a major solvent in mobile phase, and therefore it is expected that the different retention performance would be observed by the use of heavy water as a mobile phase solvent.

Table-3 Retention data for alkylbenzenes with C-2, C-8 and C-18 columns.

(A) C-2 column.

sample	CH_3OH/D_2O k'	α	CH_3OH/H_2O k'	α	CH_3CN/D_2O k'	α	CH_3CN/H_2O k'	α
benzene	0.84	-	0.79	-	1.69	-	1.48	-
toluene	1.15	1.37	1.03	1.30	2.51	1.49	2.12	1.44
ethylbenzene	1.52	1.80	1.38	1.74	3.53	2.09	3.03	2.05
n-propylbenzene	2.18	2.60	2.00	2.53	5.14	3.04	4.53	3.07
n-butylbenzene	3.12	3.72	2.86	3.62	7.41	4.41	6.64	4.50
n-amylbenzene	4.64	5.52	4.26	5.40	11.1	6.57	9.79	6.64
composition	7/3				1/1			

(B) C-8 column.

benzene	1.07	-	1.04	-	1.16	-	1.05	-
toluene	1.44	1.35	1.38	1.33	1.49	1.29	1.34	1.27
ethylbenzene	1.91	1.79	1.82	1.75	1.91	1.64	1.72	1.63
n-propylbenzene	2.77	2.59	2.58	2.48	2.53	2.18	2.27	2.15
n-butylbenzene	4.14	3.87	3.79	3.64	3.31	2.86	3.02	2.86
n-amylbenzene	6.27	5.86	5.62	5.40	4.39	3.78	3.97	3.76
composition	3/2				1/1			

(C) C-18 column.

benzene	1.05	-	1.05	-	1.22	-	1.12	-
toluene	1.67	1.59	1.55	1.48	1.64	1.34	1.45	1.30
ethylbenzene	2.25	2.14	2.14	2.05	2.12	1.73	1.89	1.69
n-propylbenzene	3.48	3.31	3.18	3.04	2.90	2.37	2.63	2.35
n-butylbenzene	5.32	5.06	4.84	4.63	3.98	3.25	3.66	3.27
n-amylbenzene	8.30	7.89	7.46	7.14	5.55	4.53	5.16	4.61
composition	7/3				3/2			

α is referred to benzene.

Column; 0.5 mm i.d. x 12 cm, C-18(FineSIL)

Chromatographic conditions: flow-rate; 8 μL/min

column temperature; 25°C.

In Table-4, the capacity factors and the separation factors of noradrenalin, adrenalin and dopamine are listed. It is apparent that the retention factors with heavy water seems to be rather agreeable. Although preferable separation was attained with heavy water, the same or similar effect could have been obtained by changing the chromatographic conditions, e.g., column temperature and dimension, packing materi-

Table 4 Retention data for catecholamines with heavy water and light water as mobile phase.

sample	retention factor			
	heavy water		light water	
	k'	α	k'	α
noradrenalin	0.85	—	0.57	—
adrenalin	1.81	2.13	1.19	2.09
dopamine	3.20	3.77	2.05	3.61

Chromatographic conditions:
column; 0.5 mm i.d. x 12 cm length, SC-01
flow-rate; 16 μL/min, 0.1 M acetic acid buffered.
column temperature; 25°C
α is referred to noradrenalin.

als, flow-rate of mobile phase, etc.. So these behaviors of heavy water may be of little real benefit.

A chloroform solution of the test mixture of four diethyldithiocarbamates was injected in the silica column. The chromatograms were separately measured with benzene and deuteriobenzene as the mobile phase. Then capacity factors for each metal complexes were calculated in the normal way using FC-78 as non retained substance.

The results are shown in Table 5. The capacity factors with deuteriobenzene mobile phase are about 10 % smaller than those with benzene, although the theoretical mean of this fact is not cleared yet. The separation performance of deuteriobenzene is inferior to that of benzene. However, it is apparent that the slightly faster separation would be possible by using deuterated solvent as mobile phase.

The observed isotope effects on chromatographic performance are considered to be caused from the reasons as follows;
deuterated solvents such as heavy water and deuteriobenzene have the same electronic structures as light water and benzene, but the higher

Table 5 Retention data for metal complexes with benzene and deuteriobenzene as mobile phase.

sample	retention factor			
	benzene		deuteriobenzene	
	k'	α	k'	α
Cu-complex	0.39	—	0.41	—
Cr-complex	0.95	2.45	1.05	2.56
Co-complex	1.31	3.38	1.47	3.55
Fe-complex	1.57	4.07	1.73	4.20

Chromatographic conditions:
column; 0.5 mm i.d. x 15 cm, silica.
flow rate; 8 μL/min
column temperature; 25°C
α is referred to Cu-complex.

mass of deuterium restricts their nuclear motions relative to hydrogen, making deuterated molecules more structured. Therefore it is expected that deuterated solvents behave as chromatographically more "polar" in nature when compared with the normal substances, since the more structured deuterated molecules would give still tighter packing on the basis of hydrophobic effects associated with tight packing of normal molecules around the solute. This explanation has been confirmed in many publications (20-23), in which separations of normal and deuterated substances were carried out in reversed phase mode and deuterated solutes eluted faster than normal solutes, because tight packing of water molecules about the solute and of specific solvation of C-H bonds (24,25) might explain the more restricted motion in the aqueous phase relative to the hydrophobic phase that is indicated by the isotope effects, which relatively favor deuterium over protium in the aqueous phase.

The potential of the use of deuterated solvents as mobile phase is expected in combination of liquid chromatography and infrared spcetrometry (IR).

Although the two detectors based on the UV absorption and refractive index (RI) are the most often used, they provide little structural information on the separated components. IR is undoubtedly one of the most useful tools providing quantitative information. The large number of absorption bands present in an IR spectrum offer specific information of functional groups, possible structure assignments and confirmation of postulated structure.

Many efforts have so far been made to combine HPLC with IR spectrometry on-line (26-28). One of them is a simple IR photometer designed for use a HPLC/IR detector with a flow-cell manufactured by Foxboro/Wilks (29). The use of this kind of IR detector is however limited to the mobile phases that are transparent at the wavelength of interest. In most cases at least 30 % transmission of the solvent with the cell enough required on the pathlength is needed for satisfactory application. Unfortunately, most of practical HPLC solvents have strong bands in mid-IR regions. The experimental results, in which deuterated solvents affect the solute selectivity only marginally, indicate that HPLC/IR combination via direct flow-cell technique can be easily accomplished free from mobile phase absorption interference. For instance, Figure-1 shows the infrared spectrum of benzene and deuteriobenzene measured by FT-IR. It is appeared that the absorption of C-H stretching frequencies of any solute can be detected because of the shift of $\nu_{CH} - \nu_{CD}$. The second example in Figure-2 is the IR spectrum of light water, heavy water and PTFE on-column flow-cell (15). The detection of components which have O-H bonds in their structures can be attained without any change of chromatographic conditions by the use of heavy water as mobile phase instead of light water, since the isotope shift of the $\nu_{OH} - \nu_{OD}$ eliminates the O-H absorption of aqueous mobile phase. As shown in

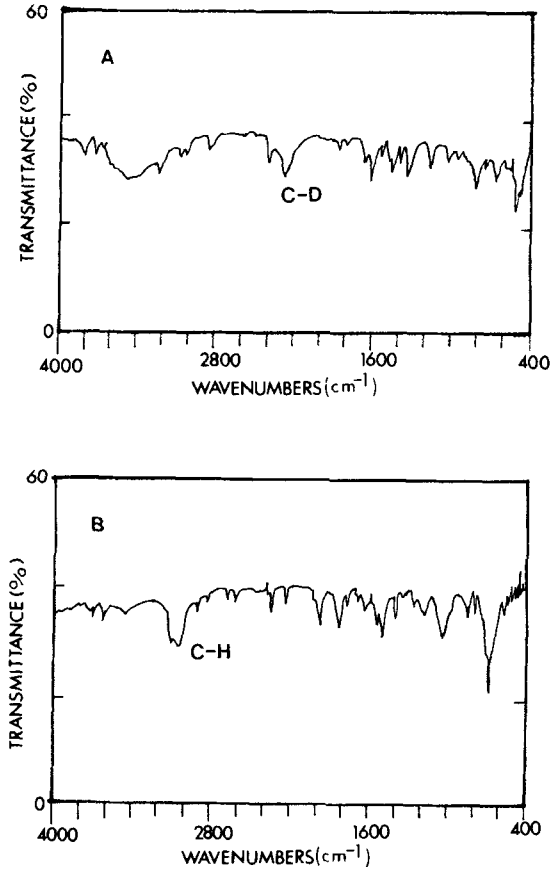

Figure 1 Infrared Spectra of Benzene and Deuteriobenzene.

A: Benzene
B: Deuteriobenzene
FT-IR conditions: 1 drop on a KBr disc.
resolution; 4 cm^{-1}.
accumulation; 10 times.
detector; TGS.

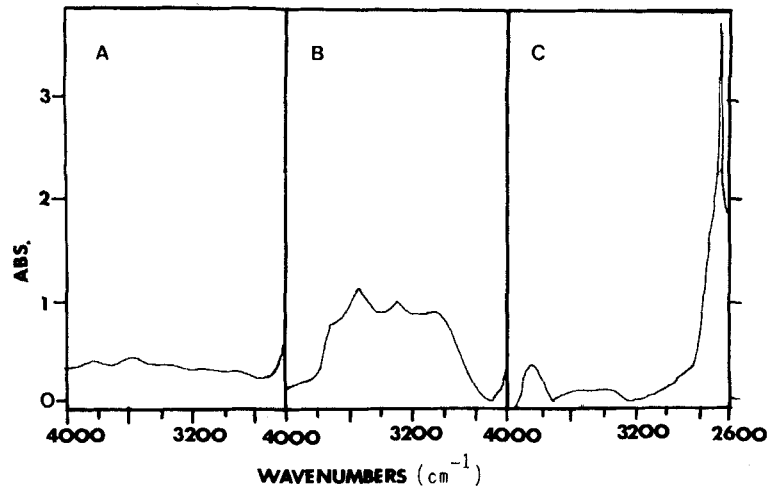

Figure 2 Infrared Spectra of (A) PTFE on-column flow-cell, (B) light water and (C) heavy water.
FT-IR conditions: on-column flow-cell with a beam condensor.
resolution; 16 cm^{-1}
accumulation; 10 times.
detector; MCT.

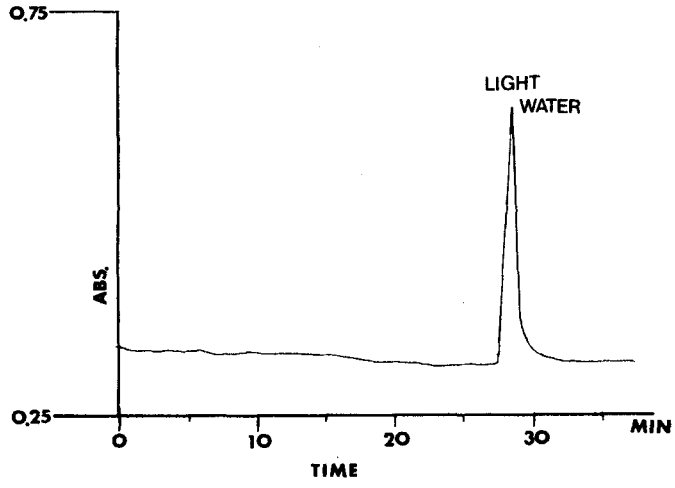

Figure 3 FT-IR Chromatogram of Water by Size Exclusion Mode.

column: FineGEL SC-220, PTFE tubing of 0.65 mm i.d. x 44 cm.
mobile phase: heavy water.
flow-rate: 4 µL/min.
sample amount: 0.5 µL.
FT-IR conditions: resolution; 16 cm^{-1}.
accumulation; 10 times.
detector; MCT.

Figure-3, this approach clearly demonstrates that trace water in heavy water could be measured with FT-IR on monitoring at 2950 cm^{-1} chromatographically.

This concept can be also applied to the combination of micro-HPLC and proton-NMR free from interference of protons belongs to mobile phase solvents.

In the combination of micro-HPLC and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP) (30-32), deuterated solvents will promise the possibility of monitoring of the emission of hydrogen atom contained in solutes, because the emission line of deuterium atom is measured at 656.1 nm, while that of hydrogen atom is 656.3 nm. The use of high resolution monochromator can resolve both lines as separated.

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