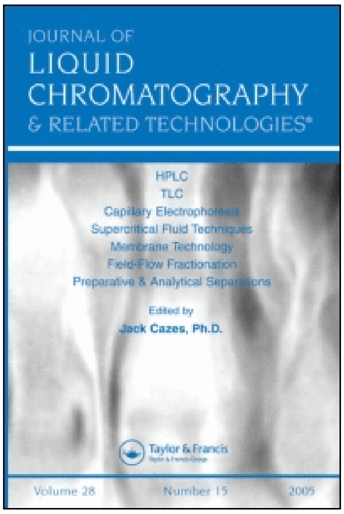

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Studies on Iodinated Compounds. IV. High Performance Liquid Chromatographic Determination of Iodide with a Complex of Primary Ammonium Ion - Crown Ether

Mashiro Miyashita[a]; Saburo Yamashita[a]
[a] Department of Clinical Chemistry, Hoshi College of Pharmacy, Shinagawaku, Tokyo

To cite this Article Miyashita, Mashiro and Yamashita, Saburo(1986) 'Studies on Iodinated Compounds. IV. High Performance Liquid Chromatographic Determination of Iodide with a Complex of Primary Ammonium Ion - Crown Ether', Journal of Liquid Chromatography & Related Technologies, 9: 10, 2143 — 2151

To link to this Article: DOI: 10.1080/01483918608074139

URL: http://dx.doi.org/10.1080/01483918608074139

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(10), 2143-2151 (1986)

STUDIES ON IODINATED COMPOUNDS. IV. HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF IODIDE WITH A COMPLEX OF PRIMARY AMMONIUM ION - CROWN ETHER

Mashiro Miyashita and Saburo Yamashita
Department of Clinical Chemistry
Hoshi College of Pharmacy
2-4-41 Ebara, Shinagawaku, Tokyo 142

ABSTRACT

Determination of iodide (I^-) was performed using a complex of primary ammonium ion - crown ether as the counter ion on reversed-phase high performance liquid chromatography (HPLC). Retention of the I^- in the present system increased more than the corresponding counter ion system, using the only primary ammonium ion. The rate of increase of the retention was dependent on pHs', concentration of crown ether and organic solvents of the eluent. Determination of I^- was investigated with hexylamine and 18-crown-6 with a UV detector at 225 nm. The calibration curve was linear from 25 pmoles to 25 nmoles, and the least detection of I^- was 1 pmole.

INTRODUCTION

Iodide (I^-) was determined with high sensitivity on the high performance liquid chromatography (HPLC) by separation with

0148-3919/86/0910-2143$3.50/0

reversed-phase ion pair partition methods and detection with a UV detector (1-4). In the above system, amines were used as the counter ions in the eluent, and primary, secondary amines and even quarternary ammonium ion could be used for such purposes.

On the other hand, crown ethers, which are known to form complex with cations, have been used in the HPLC system (5). The authors also tried to apply some crown ethers in the determination of I^-; a crown ether, 18-crown-6 (18-C-6), was added to the amine containing eluent. It was found that when an eluent containing primary amine and 18-C-6 was used, retention of I^- increased more than primary amine alone. The fact might be due to the formation of complex between the primary amine (primary ammonium ion) and crown ether (6). In other words, hydrophobic property of the crown ether might contribute to the nature of the counter ion. Thus formed more hydrophobic counter ion increased retention of I^-. Interesting thing is that secondary, tertiary or quaternary ammonium ions with 18-C-6 in the eluent did not alter the retention of I^- under the same conditions as in the case of primary amine. The fact may be due to that the amines except primary ones do not form such complex with crown ethers (6).

In the present paper, we have investigated the separation and determination of I^- on HPLC using primary ammonium ion - crown ether complex as the counter ion.

MATERIALS AND METHODS

Reagents

Hexylamine and 18-crown-6 were the extra pure reagent grade reagents obtained from Nakari Chem. Co.. KI, butylamine and octylamine were the guaranteed grade reagents obtained from Wako Pure Chem. Co.. Other reagents were the analytical grade ones. Water used as the eluent on HPLC was obtained by re-distilling ion-exchanged water. Acetonitrile (MeCN), methanol (MeOH) and tetrahydrofuran (THF) were HPLC grade obtained from Kanto Chem. Co..

Instruments

HPLC system was composed of Waters' Type 6000A pump and universal U6K injector, and variable wave length UV detector Type NS-310A of Nippon Seimitsu Kagaku Co.. HPLC column was Radial Compression Separation System of Waters' and a cartridge Radialpak Novapak C_{18} (8 mm i. d. x 100 mm, 5 µm) was used.

Determination

Eluent (15% MeCN / 5 mM hexylamine phosphate (pH 4.0) + 5mM 18-C-6) was prepared as follows: To 0.51 g (5 mmoles) of hexylamine, 980 ml of distilled water was added to dissolve and pH of the solution was adjusted to 4.0 with phosphoric acid, and the final volume was adjusted to 1000 ml with distilled water. Then, 1.32 g (5 mmoles) of 18-C-6 was dissolved in the solution, and thus made solution was added to 150 ml of MeCN up to 1000 ml. The solution was filtered through a 0.45 µm filter. Other eluents were prepared by the similar procedures.

HPLC was performed at room temperature (20 - 23 °C) with 2.5 ml / min flow rate, and I^- was detected at 225 nm.

For the purpose of the determination of the capacity factor, k', the hold up volume of the column was obtained with $NaNO_3$ eluted with 60% MeCN.

RESULTS AND DISCUSSION

Effect of PH of the Eluent

The retention (k') of I^- at various pHs' (3.0 to 7.0) was investigated with the eluent containing 5 mM butylamine, hexylamine or octylamine with or without 5 mM 18-C-6 (Fig. 1).

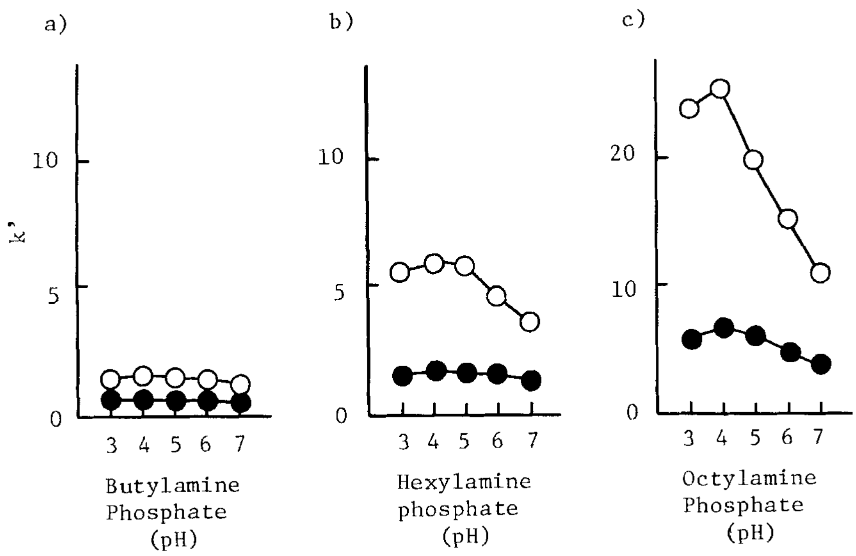

FIGURE 1 Effect of PH on Retention of I^-

Eluent: ○ ; 15% MeCN / 5 mM Butyl- (Hexyl- or Octyl-) amine Phosphate + 5 mM 18-Crown-6,
● ; 15% MeCN / 5 mM Butyl- (Hexyl- or Octyl-) amine Phosphate

In any case, k' of I^- increased by the addition of 18-C-6. The k' value of I^- in the eluent consisting of amine and 18-C-6 increased as the pH became lower, reaching at the maximum k' at pH 4.0. Changing profile of the retention of I^- was more distinctly altered by amines with longer carbon-chain.

One of the reasons of the pH dependency with crown ether may be that the complexes of the amines and crown ether are more stable at lower pH region. However, when octylamine was used without crown ether, almost the same retention behavior was observed as hexylamine used with crown ether. The fact may be due to that the pH dependency with crown ether is ascribable to primary amines and not to the crown ether. Addition of crown ether merely gives the same effect as the extention of carbon-chain length of amines.

Effect of Concentration of Crown Ether in the Eluent

Various amounts of the crown ether 18-C-6, from 2.5 mM to 10.0 mM, were added to 5 mM hexylamine, and the retention of I^- was investigated (Fig. 2). The k' of I^- increased as the amount of 18-C-6 increased.

Effect of Organic Solvents in the Eluent

Effect of organic solvents (MeOH, MeCN, THF) in the eluent and their concentration on the retention of I^- were investigated. As the ratio of organic solvent increased, retention of I^- decreased. When MeOH or MeCN was used together with 18-C-6, retention of I^- increased always, but when THF was used with 18-C-6, such tendency was not observed. THF is known to have some affinity with cations (7), and it may form complexes with amines. Therefore, THF and 18-C-6 may form complexes with amines competitively, while MeOH and MeCN do not have such chemical properties.

Calibration Curve and Reproducibility

Quantitative determination of I^- on the HPLC was performed using 15% MeCN / 5 mM hexylamine phosphate (pH 4.0) + 5 mM 18-C-6, as the eluent. Wave length for the detection of I^- was set at the maximum wave length, 225 nm ($\varepsilon = 1.47 \times 10^4$). The calibration curve was prepared as follows: the standard solution of KI, 10^{-5} M, 10^{-4} M and 10^{-3} M were injected from 2.5 µl to 25 µl at 0.01, 0.1 and 1.0 AUFS (absorbance unit full scale) respectively, and the obtained peak heights were plotted. The regression formulae by the least-squer method are obtained (Table 1). The curves are linear intersecting near the original point.

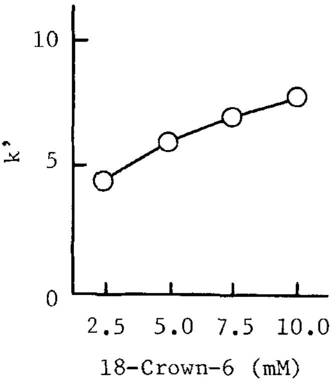

FIGURE 2 Effect of 18-Crown-6 Concentration on Retenrion of I^-

Eluent: 15% MeCN / 5 mM Hexylamine Phosphate (pH 4.0) + 18-Crown-6

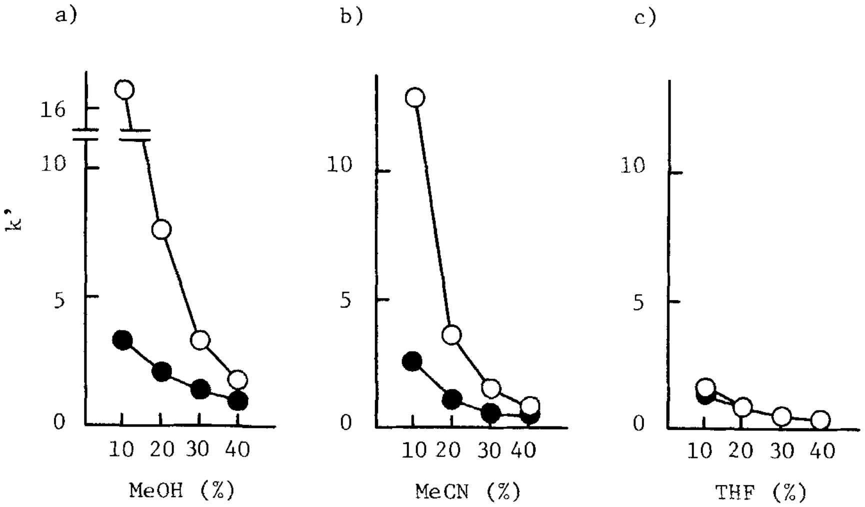

FIGURE 3 Effect of Organic Solvent on Retention of I^-

Eluent: -○- ; MeOH (MeCN or THF) / 5 mM Hexylamine Phosphate (pH 4.0) + 5 mM 18-Crown-6,
-●- ; MeOH (MeCN or THF) / 5 mM Hexylamine Phosphate (pH 4.0)

TABLE 1

Regression Lines of the Calibration Curves for I^-

Detector Sensitivity (AUFS)	I^- Level (nmoles)	Regression Line	Correlation Factor
0.01	$2.5 \times 10^{-2} \sim 2.5 \times 10^{-1}$	$Y = 5.61 \times 10^{1} X + 1.57 \times 10^{-1}$	0.9987
0.1	$2.5 \times 10^{-1} \sim 2.5 \times 10^{0}$	$Y = 6.10 \times 10^{0} X - 2.70 \times 10^{-1}$	0.9999
1.0	$2.5 \times 10^{0} \sim 2.5 \times 10^{1}$	$Y = 5.45 \times 10^{-1} X - 2.39 \times 10^{-1}$	0.9999

Y: Peak Height (cm), X: I^- (nmoles)

KI standard solution, 10 µl, was repeatedly injected at each detection sensitivities (n = 5), and the coefficients of variation corresponding to each peak heights were obtained: 0.67% at 0.01 AUFS, 0.92% at 0.1 AUFS, 0.85% at 1.0 AUFS, which were shown to be satisfactory reproducibility.

The least detection at 0.005 AUFS was 1 pmol (S / N = 3) of I^-.

Effect of Different Ions

Effect of other cations than I^- was investigated. Such cations as 100 nmoles KOH, NaOH or NH_4OH were added to 1 nmole KI, and the mixture was injected into HPLC system, and the obtained chromatograms were compared. No significant change in the retention of I^- was shown, and the peak heights with and without cations were within ± 3% of difference, so that disturbance by the cations might be negligible. On the other hand, effects of other halogen ions were examined using 100

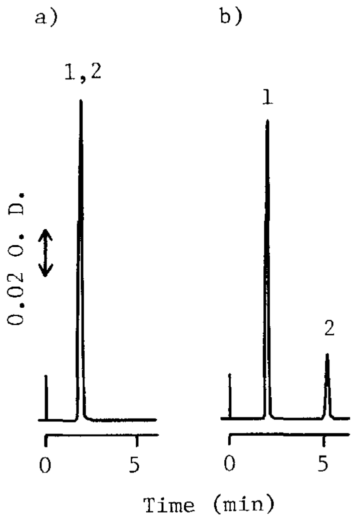

FIGURE 4 High Performance Liquid Chromatogram of Iodouracil and I^-

Eluent: a) 15% MeCN / 5 mM Hexylamine Phosphate (pH 4.0),
b) 15% MeCN / 5 mM Hexylamine Phosphate (pH 4.0) + 5 mM 18-Crown-6
Sample: Iodouracil (1.25 μg) + KI (0.25 μg)
Peak: 1; Iodouracil, 2; I^-

nmoles KF, KCl or KBr, but these halogens did not disturb the determination of I^- on HPLC under the conditions indicated here.

Application of the Method to the Determination of I^- as Impurity

Thus developed determination method of I^- on HPLC was applied for the quality control of free I^- in commercially available organic iodide compounds. The sample organic iodide was iodouracil, manufactured and sold by Nakarai Chem. Co.. HPLC chromatogram of standard iodouracil and I^- is shown in Fig. 4. Fig. 4-a) shows separation of iodouracil and I^- eluted with a solvent containing no crown ether, and the both peaks were not separated. When the crown ehter was added to this solvent, retention of I^- was selectively increased, and thus the both peaks could be separated sufficiently (Fig. 4-b)). The iodouracil preparation, 5 µg applied on HPLC, used in the present study contained no free I^-.

Conclusion

The present study demonstrated usefullness of the crown ether in the determination of I^-; that is, the complex of primary ammonium ion and the crown ether could be used for the determination of the I^- satisfactorily. Sensitivity and reproducibility of the determination were comparable to the conventionally used HPLC method without the crown ether.

References

1. Skelly, N. E., Separation of Inorganic and Organic Anions on Reversed-Phase Liquid Chromatography Columns, Anal. Chem., 54, 712, 1982.

2. Hurst, W. J., Snyder, K. P. and Martin, Jr., R. A., The Determination of Iodine in Milk and Milk Chocolate by Anion HPLC, J. Liq. Chromatogr., 6, 2067, 1983.

3. Miyashita, M., Kaji, K., Seyama, Y. and Yamashita, S., Studies on Iodinated Compounds. III. High-Performance Liquid Chromatographic Determination of Iodide (I^-), Chem. Pharm. Bull., 32, 2430, 1984.

4. Nagashima, H., Kuboya, K. and Onogiku, S., 1st Conference on Ion Chromatography, Saitama, 1984, p. 13.

5. Shono, T., Crown Ethers in Analytical Chemistry, Bunseki Kagaku, 33, E449, 1984.

6. Hiraoka, M., Yanagida, H., Ohara, M. and Koga, K., Host-Guest Chemistry, Kodansya, Tokyo, 1984, p. 134.

7. Hiraoka, M., Crown Compounds, Kodansya, Tokyo, 1978, p. 12.