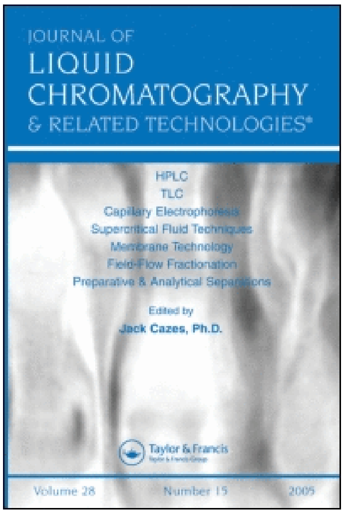

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CHEMICALLY REMOVABLE DERIVATIZATION REAGENT FOR CHROMATOGRAPHY. II. 2-(1-NAPHTHYL)ETHYL 2-[1-(4-BENZYL)-PIPERAZYL]ETHANESULFONATE DIHYDROCHLORIDE

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ABSTRACT

A sulfonate reagent, 2-(1-naphthyl)ethyl 2-[1-(4-benzyl)piperazyl]ethanesulfonate dihydrochloride, was synthesized for analytical derivatization in liquid chromatography. The reagent has two main functions, one with a chromophore (naphthyl) for detection and the other with a substituted piperazine moiety for being removable after derivatization. The reagent was preliminarily applied to the derivatization of iodide anion. The results indicated that the reagent can be readily removed by acid after derivatization; this favorably avoids the interference of the excess reagent with the resulting iodide derivative for analysis.

INTRODUCTION

Analytical derivatization coupled with chromatography[1-4] has found a wide range of application in the fields of chemical and biochemical sciences. Based on analytical derivatization, a highly responsive tag such as chromophore, electrophroe or fluorophore can be incorporated with an analyte for trace analysis. Usually, much higher concentration of a derivatization reagent as compared to that of an analyte is required for the derivatization of the analyte at trace levels. As a consequence, the excess reagent may seriously interfere in the separation of the target derivative for sensitive detection, especially in case of a derivatization reagent itself also being very responsive to a detector. To solve this problem, common approaches used includes the removal of excess reagent by nitrogen purge[5] and by additional column clean up[6]; these treatments are usually tedious and time consuming. Therefore, attempts for devising the derivatization reagents with removable properties after reaction were made and resulted in the syntheses of 2-(N-phthalimido)ethyl 2-(dimethylamino)ethanesulfonate[7] and 2-(1-naphthyl)ethyl 2-[1-(4-benzyl)piperazyl]ethanesulfonate dihydrochloride (NEBPES • 2HCl) (Fig. 1). Using iodide as a model analyte, NEBPES can derivatize iodide into a chromopheric derivative, 2-(1-naphthyl)ethyl iodide in a biphasic system, for chromatographic analysis. The excess reagent, can be easily removed as water soluble salt after derivatization by protonation (acid treatment) of the tertiary nitrogens of its piperazine moiety. This presents a simple approach for the removal of the unwanted reagent for the analytical derivatization.

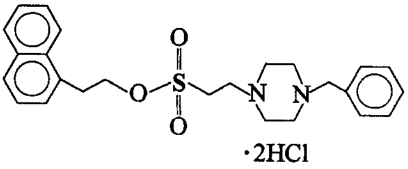

FIGURE 1. Structure of 2-(1-naphthyl)ethyl 2-[1-(4-benzyl)piperazyl]ethanesulfonate dihydrochloride

METHODS

Materials and Reagent

Benzyldimethylphenylammonium chloride (BDMPAC), 2-(1-naphthyl)ethanol, 2-chloroethanesulfonyl chloride, N-benzylpiperazine and 1,2,4,5-tetrachlorobenzene (TCB) (TCI, Tokyo, Japan), tetra-n-amylammonium chloride (TAAC) (Wako, Osaka, Japan), tetra-n-butylammonium chloride (TBAC) (Fluka, Buchs, Switzerland), tetra-n-butylammonium hydrogen sulfate (TBAHS) (Aldrich, Milwaukee, USA), trimethylamine (45%, w/v), toluene, dichloromethane, methanol, sodium carbonate, potassium hydroxide, potassium iodide, hydrochloric acid (37%, w/v), sulfuric acid and silica gel 60 (70-230 mesh) (E. Merck, Darmstadt, Germany), acetonitrile (Fisher, Fair Lawn, NJ, USA) were used without further treatment. All other chemicals were of analytical-reagent grade. Solution of TCB was prepared by dissolving the appropriate amounts of the compound in toluene. Solutions of potassium iodide, potassium hydroxide, sodium carbonate, NEBPES • 2HCl, TAAC, TBAC, TBAHS and BDMPAC were prepared by dissolving the appropriate amounts of the respective compounds in distilled and deionized water.

HPLC Conditions

A Waters-Millipore LC system with a U6K injector, a Model 510 pump and a Model 486 UV-Vis detector was used. A Nova-Pak C_{18} column (150 x 3.9 mm I.D., 4 μm) and a mobile phase consisting of 65% (v/v) acetonitrile in water at a flow-rate of 0.8 mL/min were used. The column eluate was monitored at 282 nm. The solvents were pretreated with a vacuum filter for degassing.

Synthesis of 2-(1-Naphthyl)ethyl 2-[1-(4-Benzyl)piperazyl]-ethanesulfonate Dihydrochloride (NEBPES • 2HCl)

1. Synthesis of 2-(1-Naphthyl)ethyl Ethenesulfonate (NEES)

2-(1-Naphthyl)ethanol (8.61g, 50.00 mmol), 2-chloroethanesulfonyl chloride (15.78 mL, 150.04 mmol) and trimethylamine (31.00 mL, 200.00 mmol) were added successively to a 100-mL reaction flask containing 50.00 mL of dichloromethane pre-cooled in an ice bath. The solution was magnetically stirred at 0℃ for 2 h, then the resulting mixture was washed with cold sodium carbonate solution (10%, v/v) (50 mL × 2) and water (50 mL). The separated organic layer was dried with anhydrous sodium sulfate (ca. 2.50 g) and the filtrate was concentrated by a rotary evaporator. The liquid residue was purified by column chromatography (40 × 4 cm I.D.) on silica gel 60 (ca. 200 g) with ethyl acetate - n-hexane (1:3, v/v) as an eluent to give NEES (8.02 g, 30.61 mmol, 61.22% yield) as colorless oily liquid. Element analysis (%) calculated for $C_{14}H_{14}O_3S$: C, 64.12; H, 5.35; O, 18.32; found: C, 64.07; H, 5.47; O, 18.25. MS (EI): m/z 262 (M^+); 141 (naphthylmethyl), a basal peak.

2. Synthesis of NEBPES • 2HCl

NEES (7.86 g, 30.00 mmol) and N-benzylpiperazine (6.34 g, 36.04 mmol) were added to a 150-mL reaction flask containing dichloromethane (40.00 mL) pre-cooled in an ice bath. The mixed solution was magnetically stirred at 0℃ for 0.5 h, then the resulting solution was concentrated by a rotary evaporator. The liquid residue obtained was purified by column chromatography (40 × 4 cm I.D.) on silica gel 60 (ca. 200 g) with ethylacetate - n-hexane (1:2, v/v) as an eluent, to give NEBPES (11.56 g, 26.40 mmol, 88.00% yield) as colorless oily liquid, ^{1}H NMR ($CDCl_3$): δ 2.35 (br, 8H, piperazyl H); 2.67 (t, 2H, N-$\underline{CH_2}$-CH_2-SO_2); 3.10 (t, 2H, N-CH_2-$\underline{CH_2}$-SO_2); 3.45 (s, 2H, N-$\underline{CH_2}$-phenyl); 3.51 (t, 2H, $\underline{CH_2}$-naphthyl); 4.53 (t, 2H, CH_2-$\underline{CH_2}$-OSO_2); 7.29-8.02 (m, 12H, aromatic H). Analysis (%) calculated for $C_{25}H_{30}N_2O_3S$: C, 68.49; H, 6.85; N, 6.39; O, 10.96; found: C, 68.28; H, 6.97; N, 6.27; O, 10.90. MS (FAB): m/z 439 ($M^+ + 1$); 347 (M^+ minus benzyl); 283 (M^+ minus naphthylethyl); 203 [1-(4-benzyl)piperazylethyl], a basal peak; 155 (naphthylethyl); 91 (benzyl).

NEBPES • 2HCl was prepared by adding hydrochloric acid (12 mL, 121.64 mmol) gradually to a cold solution of NEBPES (10.95 g, 25.00 mmol) in methanol (100 mL). The resulting mixture was concentrated and the residue obtained was recrystallized from ethanol - n-hexane (8:1, v/v) to give a white needle crystal (8.85 g, 17.32

mmol, 69.28% yield), m.p. 183.8-184.5℃ ; analysis (%) calculated for $C_{25}H_{32}N_2O_3SCl_2$: C, 58.71; H, 6.26; N, 5.48; O, 9.39; found: C, 58.91; H, 6.38; N, 5.52; O, 9.54.

Derivatization Procedure

A 0.1-mL aliquot of iodide solution (0.25 μM) was added to a 10-mL glass stoppered test-tube containing 0.10 mL TAAC solution (PTC) (0.20 M) and 0.50 mL TCB solution (I.S., 0.37 μM), then 0.70 mL NEBPES • 2HCl solution (28.57 μM) and 0.10 mL potassium hydroxide solution (0.50 M) were added. The reactants were shaken at 95℃ for 2 h. After cooling, the reaction mixture was added with 1.0 mL of H_2SO_4 aqueous solution (1.00 M) and the test tube was vortexed for 30 s. An aliquot of the toluene layer (0.10 mL) was five-fold diluted with acetonitrile for compatibility with reversed-HPLC. The resulting solution was used for HPLC analysis (25 μL).

RESULTS AND DISCUSSION

The chemical removability and reactivity of the new reagent (NEBPES • 2HCl) were studied, using iodide anion as a test model. For optimization of the derivatization conditions for iodide at an amount of 25 nmol, several parameters including reaction solvent, phase-transfer catalyst (PTC), concentrations of KOH, molar ratio of NEBPES • 2HCl to iodide, reaction temperature and reaction time were evaluated by measuring the peak-area ratio of the iodide derivative to the I.S.

Removability of Derivatizing Reagent after Derivatization

NEBPES • 2HCl was used in large excess to derivatize iodide (25 nmol), equivalent to a molar ratio of about 800 as indicated in the derivatization procedure. After derivatization, the reaction mixture was either treated with or without H_2SO_4 solution (1.00 mL, 1.00 M). The results are shown in Fig. 2. A broad tailing peak overlaped with the peaks of the iodide derivative and the I.S., resulting from the derivatization without further treatment with the acid solution. On the other hand, the interfering NEBPES peak can be easily removed after derivatization with a simple treatment of the reacted solution with the acid solution, favorably resulted from the protonation of the tertiary amino functions of NEBPES. This leads to a water soluble quaternary ammonium species of NEBPES; it was easily removed from the organic toluene layer after derivatization, also shown in Fig. 2.

Effect of Reaction Solvent

The effects of various organic solvents (0.50 mL) on the derivatization of iodide anion in aqueous phase (1.00 mL) were studied according to the derivatization procedure but at different temperatures in order to prevent the solvents from boiling. The immiscible solvents tested included toluene at 95℃, benzene at 70℃, chloroform at 50℃ and dichloromethane at 30℃. Toluene was found to be the best solvent for the derivatization of iodide.

Effect of Phase-Transfer Catalyst

The effects of quaternary ammonium compounds (each with 0.10 mL) at suitable concentration (based on their aqueous solubility) on

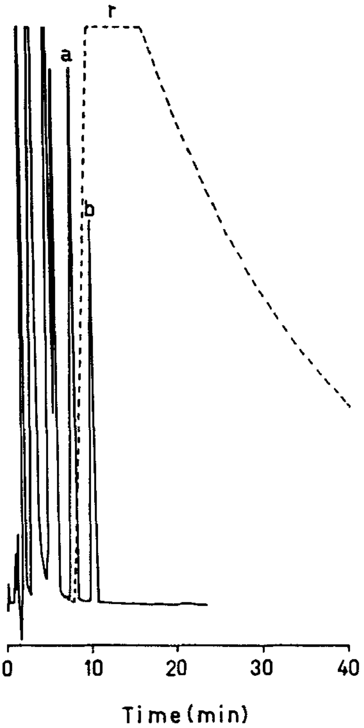

FIGURE 2. Composite liquid chromatogram of iodide (25 nmol) derivatized with NEBPES • 2HCl with acid treatment (solid line) and without acid treatment (dotted line) after derivatization: peaks a, the derivative of iodide; b, 1,2,4,5-tetrachlorobenzene (I.S.) and r, the excess reagent. See text for conditions.

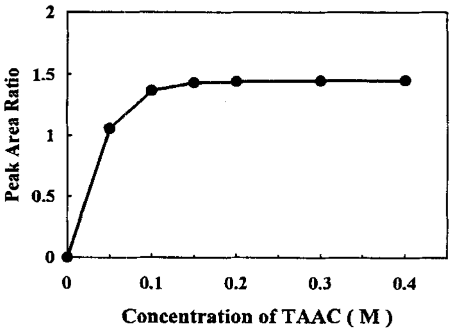

FIGURE 3. Effect of concentration of TAAC on the formation of iodide derivative. See text for conditions.

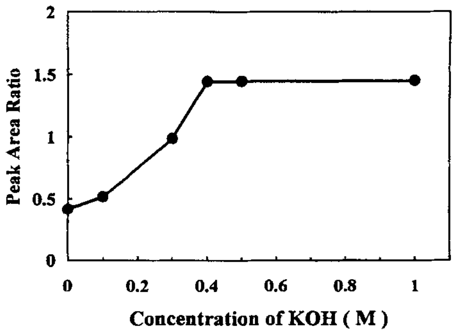

FIGURE 4. Effect of concentration of KOH on the formation of iodide derivative. See text for conditions.

the transfer of iodide from the alkaline aqueous phase to the toluene organic phase for reaction with NEBPES were cursorily studied, including TAAC (0.20 M), TBAC (0.20 M), TBAHS (1.00 M) and BDMPAC (0.10 M). The results revealed that TAAC was the best choice for the reaction system. Therefore, the effect of TAAC at various concentrations on the derivatization of iodide was further examined. The results (Fig. 3) indicated that the plateau formation of the derivative required TAAC (0.10 mL) at concentration $\geqq$ 0.15 M.

Effect of Base

Suitable amount of base (KOH) is needed to partition NEBPES • 2HCl in aqueous phase as a free amine to the organic layer for the derivatization of iodide. The effect of KOH (0.10 mL) at various concentrations on the derivatization of iodide was studied. The results (Fig. 4) indicated that the concentration of KOH were suitable over a range of 0.40-1.00 M tested.

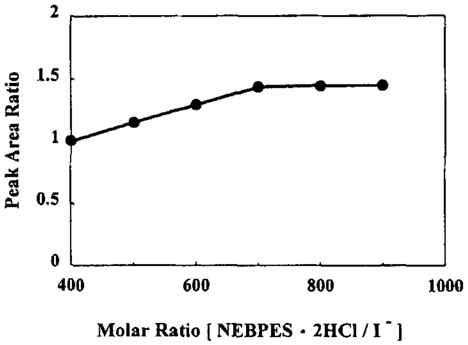

FIGURE 5. Effect of molar ratio of NEBPES • 2HCl to iodide on the formation of iodide derivative. See text for conditions.

Effect of the Derivatization Reagent

The molar ratio of NEBPES • 2HCl to iodide required for the derivatization of iodide (25 nmol) to a steady state of the derivative was studied. The results (Fig. 5) indicated that the suitable ratio is above 700.

Effect of Reaction Time

The effects of reaction time at 70 ℃ and 95 ℃ on the derivatization of iodide are shown in Fig. 6. For derivatization at 95℃, 1.5 h was needed to reach an equilibrium; with reaction at 70 ℃, plateau formation of the derivative was not attainable in 4 h and resulted in lower yields as compared with reaction at 95℃.

Analytical Calibration

Based on the optimum derivatization conditions, the derivatization procedure for iodide was formulated under the

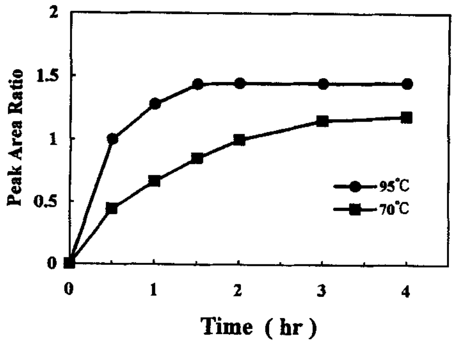

FIGURE 6. Effect of reaction time on the formation of iodide derivative. See text for conditions.

METHOD section. The quantitative application of the method to the determination of iodide was evaluated at six different amounts of iodide over the range of 0.5-25 nmol. The calibration graph was established with the peak-area ratio of the derivative to I.S. as ordinate (y) vs the amount of iodide in nmol as abscissa (x). A linear regression equation, $y = (0.0575 \pm 0.0012)x + (0.0047 \pm 0.0017)$, was obtained with a correlation coefficient 0.999 ($n = 6$), indicating good linearity of the method. The detection limit (as signal to noise ratio of 5) of iodide was about 5 pmol per injection (25 μL).

Mass Spectral Analysis of Iodide Derivative

The derivative of iodide was synthesized by scaling up the amount of iodide (1.00 mmol) with similar procedure as indicated in the derivatization procedure. The resulting derivative (colorless oily liquid) was examined by FAB-MS. The mass spectrum obtained exhibited a pseudomolecular ion of m/z 283 ($M^+ + 1$), and a basal peak of m/z 155, corresponding to a naphthylethyl moiety. This suggests that the resulting derivative is 2-(1-naphthyl)ethyl iodide. The retention time of peak a in Fig. 2 is identical to that of the synthesized derivative.

In conclusion, a sulfonate reagent NEBPES • 2HCl was synthesized and its preliminary application to the derivatization of iodide in a biphasic system was studied. The results indicated that the excess reagent can be easily removed after derivatization by a simple acid treatment, leading to the clear separation of the iodide derivative from the interferent reagent. This signals a potential approach to using chemically removable reagent for analytical derivatization. A series of sulfonate reagents with various detection-oriented tags and acid protonable functions are being developed.

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REFERENCES

1. J.F. Lawrence and R.W. Frei, Chemical Derivatization in Liquid Chromatography, Elsevier, Amsterdam, 1976.

2. K. Blau and G.S. King, Handbook of Derivatives for Chromatography, Heyden & Son, London, 1978.

3. D.R. Knapp, Handbook of analytical Derivatization Reaction, John Wiley & Son, New York, 1979.

4. F. Lawrence, R.W. Frei and J.F. Lawrence, Chemical Derivatization in Analytical Chemistry (Vol I: Chromatography), Plenum, New York, 1981.

5. J.M. Rosenfeld and J.L. Crocco, Anal. Chem., 50(6), 701-704 (1978).

6. L.G. Johnson, J. A. O. A. C., 56(6), 1503-1505 (1973).

7. H.S. Kou, H.L. Wu, Y.F. Wang, S.H. Chen and S.S. Wu, J. Chromatogr., in submission.

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