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Chemically removable derivatization reagent for liquid chromatography
I. 2-(N-Phthalimido)ethyl 2-(dimethylamino)ethanesulfonate

Hwang-Shang Kou, Hsin-Lung Wu*, Yi-Fu Wang, Su-Hwei Chen, Shin-Sheng Wu

Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical College, Kaohsiung 807, Taiwan

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Abstract

A sulfonate derivatization reagent, 2-(N-phthalimido)ethyl 2-(dimethylamino)ethanesulfonate, was synthesized and examined for use in liquid chromatography. The reagent contains two key moieties, a chromophore (phthalimido) necessary for detection and a dimethylamino function that is chemically removable after derivatization. The reagent was applied to the derivatization of 2,4,6-trichlorophenol as a model analyte. The results indicated that the reagent can be readily removed after derivatization by simple acid treatment.

1. Introduction

Analytical derivatization coupled with gas or liquid chromatography has found a wide range of applications in chemistry, biochemistry, pharmacology, toxicology, environmental science and many other disciplines; the relevant techniques for derivatization and chromatography have been extensively documented [1–6]. Basically, a large excess of derivatization reagent is used for the derivatization of an analyte at trace levels to give a derivative for sensitive analysis. Unfortunately, the excess of unreacted reagent often makes the chromatographic separation of the resulting derivative very difficult. Further disadvantages of an excess of derivatization reagent include the shortening of a column lifetime by a chemically reactive reagent and the shock to a sensitive detector caused by an intolerable input resulting from the excess of reagent.

Although several approaches have been applied for removal of excess reagent after derivatization, including using nitrogen purging for a volatile reagent [7], adding an additional chemical [8] to react with the reagent that is to be removed and column clean up [9–11]; these treatments are usually tedious, time consuming and expensive. Therefore, we sought to obtain a derivatization reagent with readily removable properties, and preliminary studies resulted in the synthesis of 2-(N-phthalimido)ethyl 2-(dimethylamino)ethanesulfonate (PEDAES). Using 2,4,6-trichlorophenol as a model organic analyte, PEDAES can be easily removed by simple acid treatment after derivatization, as illustrated in Fig. 1. This avoids interference due to reaction of the excess of the reagent with the derivative that is to be detected. The PEDAES reagent was

* Corresponding author.

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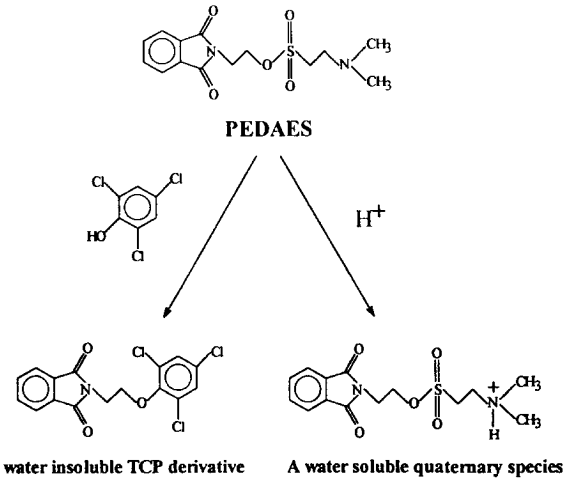

Fig. 1. Reaction scheme for PEDAES with 2,4,6-trichlorophenol and removal of excess PEDAES.

also reactive towards inorganic anions such as iodide in a biphasic water–toluene system using 18-crown-6 as the phase-transfer catalyst. The analytical derivatization of iodide will be reported elsewhere.

2. Experimental

2.1. Materials and reagents

The 2,4,6-trichlorophenol (TCP) derivative was synthesized in our laboratory and its structure was confirmed by mass and NMR spectrometry and elemental analysis. 2-Chloroethanesulfonyl chloride, N-hydroxyethylphthalimide, 18-crown-6, 4-chlorophenol (MCP), 2,4-dichlorophenol (DCP), TCP (TCI, Tokyo, Japan), dimethylamine (40%, w/v), trimethylamine (45%, w/v), toluene, potassium carbonate, sodium carbonate, sulfuric acid and silica gel 60 (70–230 mesh) (Merck, Darmstadt, Germany), diphenyl (Wako, Osaka, Japan), acetonitrile, chloroform and dichloromethane (Fisher, Fair Lawn, NJ, USA) were used without further treatment. All other chemicals were of analytical-reagent grade. Solutions of MCP, DCP, TCP, 18-crown-6 and PEDAES were prepared by dissolving the appropriate amounts in toluene and a solution of diphenyl (internal standard, I.S.) was prepared in acetonitrile.

2.2. 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} (4 μm) column (150 × 3.9 mm I.D.) and a mobile phase acetonitrile–water (55:45, v/v) at a flow-rate of 0.9 ml/min were used. The column eluate was monitored at 225 nm. The solvent was pretreated with a vacuum filter for degassing.

2.3. Synthesis of PEDAES

Synthesis of 2-(N-phthalimido)ethyl ethenesulfonate (PEES)

N-Hydroxyethylphthalimide (2.40 g, 12.55 mmol), 2-chloroethanesulfonyl chloride (2.65 ml, 25.20 mmol) and trimethylamine (7.79 ml, 50.40 mmol) were placed successively in a 100-ml reaction flask containing chloroform (60.0 ml) pre-cooled in an ice-bath. The reaction mixture was magnetically stirred at 0°C for 1.5 h, then the resulting mixture was washed successively with water (3 × 60 ml) and (10%, w/v) sodium carbonate solution (2 × 60 ml). The chloroform layer was treated with anhydrous sodium sulfate (ca. 2.5 g) and the filtrate was evaporated to dryness in a rotary evaporator. The residue dissolved in dichloromethane (4.0 ml) was purified by column chromatography (40 × 3 cm I.D.) on silica gel 60 (ca. 120 g) with dichloromethane as the eluent, to give PEES (1.48 g, 5.27 mmol) as a white powder, m.p. 87–88°C. ^{1}H NMR ($CDCl_3$): δ4.03 (t, 2H, N-CH_2, J = 5.43 Hz), 4.40 (t, 2H, CH_2-O, J = 5.46 Hz), 6.10 (d, 1H, *cis*-terminal vinyl proton, J = 9.38 Hz), 6.39 (d, 1H, *trans*-terminal vinyl proton, J = 16.64 Hz), 6.51 (dd, 1H, vinyl proton, J = 9.38 Hz), 7.72–7.90 (m, 4H, aromatic H). Analysis: calculated for $C_{12}H_{11}NO_5S$, C 51.25, H 3.91, N 4.98; found, C 51.08, H 3.90, N 4.97%. MS [fast atom bombardment (FAB)]: m/z 282 (M^+ + 1), 174

($M^+ - OSO_2CH=CH_2$), 107 (M^+ – phthalimidoethyl moiety).

Synthesis of PEDAES

PEES (1.42 g, 5.05 mmol) and dimethylamine (0.89 ml, 7.03 mmol) were placed in a 150-ml reaction flask containing dichloromethane (100 ml) pre-cooled in an ice-bath. The reaction mixture was magnetically stirred at 0°C for 1.5 h. The resulting mixture was treated with anhydrous sodium sulfate (ca. 2.5 g) and the filtrate was evaporated to dryness (1.59 g) in a rotary evaporator. An aliquot of the residue (1.0 g) recrystallized from 10 ml of *n*-hexane–chloroform (3:2, v/v) gave a colourless plate crystal (72% yield), m.p. 106–107°C. ^1H NMR ($CDCl_3$): δ2.22 (s, 6H, $N(CH_3)_2$), 2.75 (t, 2H, CH_2NMe_2, $J = 7.42$ Hz), 3.28 (t, 2H, CH_2SO_2, $J = 7.43$ Hz), 4.04 (t, 2H, CH_2N-phthalimido, $J = 5.31$ Hz), 4.50 (t, 2H, CH_2O, $J = 5.34$ Hz), 7.72–7.90 (m, 4H, aromatic). ^{13}C NMR ($CDCl_3$) from HETCO ($^{13}C/^1H$); δ37.25 [$CH_2N(CO)_2$], 45.00 (N-Me_2), 48.81 (CH_2SO_2), 52.84 (CH_2NMe_2), 65.56 (CH_2O), 123.50 and 134.24 (aromatic C other than that of angular C), 131.91 (angular aromatic C), 167.87 (imido C). Analysis: calculated for $C_{14}H_{18}N_2O_5S$, C 51.51, H 5.57, N 8.58, S 9.83; found, C 51.49, H 5.56, N 8.61, S 9.67%. MS [electron impact (EI)]: *m/z* 326 (M^+), 173 (phthalimidoethyl – H), 160 (phthalimidomethyl), 71 ($CH_2=CHNMe_2$), 58 (CH_2NMe_2).

2.4. Derivatization procedure

A 0.2-ml aliquot of TCP (50 μM) or other chlorophenol solution was added to a 10-ml screw-capped test-tube containing about 50 mg of potassium carbonate, then 0.1 ml of 18-crown-6 solution (100 mM) and 0.5 ml of PEDAES solution (60 mM) were added. The reaction mixture was shaken at 95°C for 4.0 h. After cooling, 0.4 ml of the reacted toluene solution was taken and washed with 1.0 ml of 1.0 M H_2SO_4 by vortex mixing for 30 s. A 0.1-ml aliquot of the acid-washed toluene layer was transferred into a test-tube and purged with a gentle stream of nitrogen to near dryness, then 0.1 ml of diphenyl solution (40 μM) was added and the resulting solution was used for HPLC analysis (about 15 μl).

3. Results and discussion

To study the chemical removability and reactivity of the newly synthesized reagent PEDAES, TCP was used as a model analyte in an amount of 10 nmol. The effects of reaction temperature, reaction time and amount of PEDAES on the derivatization of TCP in toluene were studied, using 18-crown-6 (10 μmol) and potassium carbonate (ca. 50 mg) as catalyst in a 0.8-ml reaction system. The resulting effects were evaluated by measuring the peak-area ratio of the derivative with respect to diphenyl (I.S.) The optimum amounts of 18-crown-6 and potassium carbonate were found to be $\geq$5 μmol and $\geq$25 mg, respectively, in the reaction system.

3.1. Effects of reaction temperature and reaction time

The reaction temperature and reaction time required to reach an equilibrium for the TCP derivative were studied. The results indicated that 4 h were needed for derivatization at 95°C, whereas with derivatization at 70°C, plateau formation of the derivative was not attainable in 6 h and a lower yield was obtained compared with reaction at 95°C. The derivatization yields of TCP at three levels (at 95°C) were all above 95% as shown in Table 1, based on the peak-

Table 1
Derivatization yield of 2,4,6-trichlorophenol

2,4,6-Trichlorophenol tested (nmol)	Derivative found[a] (nmol)	Yield (%)
8.02	7.81 ± 0.04	97.4
4.01	3.83 ± 0.01	95.5
1.00	0.98 ± 0.01	98.0

[a] Mean ± S.D. of triplicate analyses.

area ratios of the TCP derivative to the I.S. in comparison with that of the synthesized TCP derivative to the I.S.

3.2. Effect of amount of derivatizing agent

The amount of PEDAES required for the derivatization of TCP (10 nmol) to plateau formation of the derivative was about 27.5 μmol, equivalent to a molar ratio of PEDAES to TCP of about 2750, as shown in Fig. 2; however, an excess of the reagent (30 μmol) was selected for the derivatization of TCP.

3.3. Removability of derivatizing agent after derivatization

TCP (10 nmol), DCP (10 nmol) or MCP (15 nmol) was derivatized with excess of PEDAES as described in Section 2.4. After derivatization, the reaction mixture was either treated with or without 1.0 *M* H_2SO_4 (1 ml). The results are shown in Fig. 3. A broad and large reagent peak overlapped that of the chlorophenol derivatives resulting from the derivatization without treatment with acid solution. On the other hand, the interfering PEDAES peak in Fig. 3 can be easily removed after derivatization by simple treatment of the reaction solution with H_2SO_4, based on the protonation of the tertiary amino function of PEDAES to form a water-soluble quaternary species. The resulting quaternary species in water can be easily separated from the chlorophenol derivatives in the toluene layer.

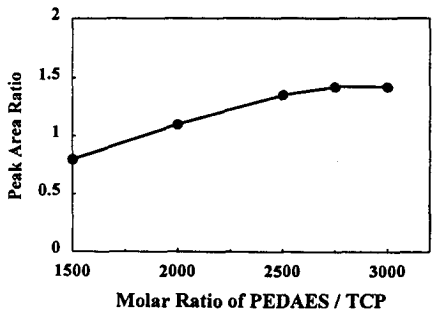

Fig. 2. Effect of molar ratio of PEDAES to 2,4,6-trichlorophenol on the formation of the TCP derivative. See text for conditions.

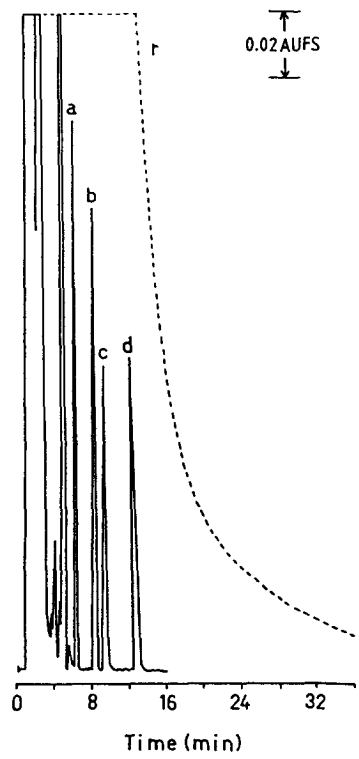

Fig. 3. Composite liquid chromatogram of 4-chlorophenol (MCP) (15 nmol), 2,4-dichlorophenol (DCP) (10 nmol) and 2,4,6-trichlorophenol (TCP) (10 nmol) derivatized with PEDAES with acid treatment (solid line) and without acid treatment (dashed line) after derivatization. Peaks: a = derivative of MCP; b = derivative of DCP; c = diphenyl (I.S.); d = derivative of TCP; r = excess of reagent. See text for conditions.

3.4. Mass spectra analysis of the derivatives

The derivative of TCP was synthesized by scaling up the amount of TCP (0.278 mmol) in toluene (20 ml) and using a similar procedure to that described in Section 2.4. The resulting derivative (m.p. 103–104°C) was examined by FAB-MS (JEOL JMX-HX 110 instrument) using thioglycerol as the sample matrix. The mass spectrum obtained exhibited a pseudo-molecular ion of *m/z* 370 (M′ = M + 1) and the typical ion peak proportions of M′: M′ + 2: M′ + 4 were equivalent to 100.0:98.0:36.0, revealing a characteristic species with three chlorine atoms, and a basal peak of *m/z* 174 was also found, corresponding to the N-phthalimidoethyl moiety.

This reasionally suggests that the resulting derivative is 2-(N-phthalimido)ethyl 2,4,6-trichlorophenyl ether. The retention time of peak d in Fig. 3 is identical with that of the synthesized derivative. The derivatives of MCP and DCP were not synthesized for retention time comparison, but peaks a and b in Fig. 3., equivalent to the derivatives of MCP and DCP, respectively, were elucidated by GC–EI-MS (Varian Star 3400 CX–Saturn 3 system). The mass spectra obtained for the derivatives of MCP and DCP exhibited their parent ions at m/z at 301 and 335, respectively, corresponding to the (N-phthalimido)ethyl ethers of the related chlorophenols.

3.5. Analytical calibration

Based on the optimum derivatization conditions for TCP as indicated in Section 2.4, the method was used to the determination of several chlorophenols, including TCP, DCP and MCP, to evaluate its quantitative applicability. The ranges of TCP, DCP and MCP levels used for the study were 0.1–10.0, 0.1–10.0 and 0.2–15.0 nmol, respectively. Five different amounts of each chlorophenol in the stated range were measured and the linearity between the peak-area ratios (y) and sample masses (x, nmol) was studied. The linear regression equations obtained were $y = (0.1423 \pm 0.0014)\ x - (0.0023 \pm 0.0002)$ with a correlation coefficient (r) of 0.999 for TCP, $y = (0.1519 \pm 0.0035)\ x + (0.0089 \pm 0.0008)$ with $r = 0.999$ for DCP and $y = (0.1012 \pm 0.0019)\ x + (0.0129 \pm 0.0009)$ with $r = 0.999$ for MCP, indicating good linearity of the method. The detection limits (signal-to-noise ratio = 5) for TCP, DCP and MCP were about 0.01, 0.01 and 0.02 nmol, respectively.

3.6. Application

The proposed method was cursorily applied to the determination of TCP spiked in water at concentrations of 50.6, 202.6 and 405.2 nM (prepared by dissolving and diluting suitable amounts of TCP in 0.01 M KOH solution). The procedure for the extraction of TCP is as follows: a 100-ml aliquot of the TCP (5.1, 20.3 or 40.5 nmol)-spiked water was acidified to pH 1 with 3 M H_2SO_4 and extracted with 1.0 ml of toluene by shaking for 5 min in a pear-shaped separating funnel. After suitable discharge of the lower water layer, a 0.2-ml aliquot of the toluene extract was directly subjected to derivatization and HPLC analysis as indicated in Section 2.4. The results are given in Table 2; the recoveries of the spiked TCP were all >92%. The method presented is very simple, using the same solvent (toluene) for the extraction and derivatization. A considerable increase in the sensitivity of the method is suggested by the extraction of a large volume of TCP-containing water sample with toluene and, in turn, concentration of the toluene extract for derivatization.

Table 2
Analytical results for 2,4,6-trichlorophenol-spiked water

Amount spiked (nmol)	Amount found[a] (nmol)	Recovery (%)	R.S.D. (%)
5.1	4.73 ± 0.07	92.7	1.48
20.3	19.12 ± 0.24	94.2	1.26
40.5	37.84 ± 0.38	93.4	1.00

[a] Mean ± S.D. of triplicate analyses.

In conclusion, the sulfonate reagent PEDAES was synthesized and its preliminary application to the derivatization of TCP, DCP and MCP was demonstrated. The results indicated that the excess of reagent can be readily removed by simple acid treatment after derivatization. This makes the separation of the resulting derivative for detection very simple. Further modification of the reagent for sensitive detection with a potential tag such as a chromophore, electrophore or fluorophore coupled with variation in the tertiary amino function for acid removal will be very attractive.

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