

Automatic Precolumn Derivatization for the HPLC Determination of Aliphatic Amines in Air

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Key Words

Volatile Aliphatic Amines, Environmental Analysis, Air Pollutant, Acclaim PolarAdvantage II Column, Fluorescence Detection

Introduction

Aliphatic amines such as methylamine, ethylamine, diethylamine, propylamine, and butylamine are considered important air pollutants due to their odorous and toxic properties. They are found in the air as a result of their industrial commercial applications and their widespread uses as intermediates in the chemical and pharmaceutical industries.¹ In addition to their toxicity, these amines have been recognized as potential precursors of carcinogenic nitrosamines in the presence of nitrous acid or other nitrosating agents in polluted air.² Therefore, determination of amines at low concentrations in the atmosphere is justified by growing concern about the public health hazards that these compounds present.

The techniques most widely used for the determination of aliphatic amines are gas chromatography (GC),^{1,3-6} high-performance liquid chromatography (HPLC),^{2, 7-13} ion chromatography,¹⁴⁻²¹ and spectrophotometry.^{22,23} Because these amines are subject to adsorption and decomposition, it is preferable to derivatize them to a stable product prior to analysis.

Precolumn chemical derivatization in solution has been accepted as an effective modification technique prior to HPLC analysis. This technique can provide highly sensitive and selective detection of these amines by bonding a chromophore or fluorophore that results in products with strong UV absorption and/or fluorescence emission. This approach can also improve retention by reducing the polarity of these compounds.

Many derivatization reagents for primary and secondary aliphatic amines prior to HPLC analysis have been reported, such as *o*-phthalaldehyde (OPA), 2, 4-dinitrofluorobenzene (DNFB), 9-fluorenylmethyl chloroformate (FMOC-Cl), dinitrochlorobenzene (DNFB), 5-dimethylaminonaphthalene-1-sulfonyl chloride (DNS-Cl), and phenylisothiocyanate (PITC).²⁴ FMOC-Cl and OPA are often used due to the rapid formation of stable derivatives under mild conditions—preferably at room temperature and in aqueous phase—and have been applied to the determination of amino acids with automatic precolumn derivatization.²⁵ Automatic precolumn derivatization eliminates tedious manual procedures, reduces error, and thereby increases method reproducibility. This technique is performed using the autosampler and controlled by the chromatography data system software.

Goal

To develop an efficient HPLC method using automatic precolumn derivatization and fluorescence detection for the determination of ethanolamine, methylamine, ethylamine, diethylamine, *n*-propylamine, *n*-butylamine, and *n*-hexylamine in air samples

Equipment

- Thermo Scientific Dionex UltiMate 3000 HPLC system, including:
 - DGP-3600RS Dual Ternary Rapid Separation Pump System with SRD-3600 Integrated Solvent and Degasser Rack
 - WPS-3000TRS Rapid Separation Wellplate Sampler, Thermostatted, with 250 μ L sample loop
 - TCC-3000RS or TCC-3000SD Thermostatted Column Compartment
 - FLD-3400RS Rapid Separation Fluorescence Detector
- Thermo Scientific Dionex Chromeleon Chromatography Data System (CDS) software version 6.80, SR9 or higher
- Thermo Scientific Orion 2-Star Benchtop pH Meter
- SK3200 KUDOS LHC Series Dual Frequency Ultrasonic Cleaner, KUDOS, Shanghai, China

Reagents and Standards

- Deionized (DI) water, 18.2 M-cm resistivity
- Methanol (CH_3OH), HPLC Grade (Fisher Chemical P/N AC610090040)
- Acetonitrile (CH_3CN), HPLC Grade (Fisher Chemical P/N AC610010040)
- Phosphoric Acid (H_3PO_4), Analytical Grade, SCRC, China
- Sodium Dihydrogen Phosphate (NaH_2PO_4), Analytical Grade, SCRC, China
- Sodium Hydroxide (NaOH), Analytical Grade, SCRC, China
- 9-Fluorenylmethyl Chloroformate (FMOC-Cl), $\geq 99.0\%$ (HPLC), Sigma-Aldrich®
- *o*-Phthalaldehyde (OPA) $\geq 99.0\%$ (HPLC), Sigma-Aldrich
- 3-Mercaptopropionic Acid $\geq 99.0\%$, Alfa, Tianjin, China
- Sodium Borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$), Analytical Grade, SCRC, China
- Ethanolamine, Methylamine, Ethylamine, Diethylamine, *n*-Propylamine, *n*-Butylamine, and *n*-Hexylamine, Analytical Grade, SCRC, China

Solutions for Derivatization

Borate buffer (400 mM, pH 10.2): dissolve 15.26 g of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ in 50 mL DI water, then dilute to 100 mL with DI water. Adjust to pH 10.2 with 1 M NaOH.

Borate buffer (20 mM, pH 10.2): dilute 5 mL borate buffer (400 mM) to 100 mL with DI water, then adjust to pH 10.2 with 1 M NaOH.

Phosphate buffer (50 mM, pH 3.0, used as injection diluent): dissolve 1.53 g of NaH_2PO_4 in 100 mL DI water, then dilute to 250 mL with DI water. Adjust to pH 3.0 with 50% H_3PO_4 .

OPA stock solution (50 mg/mL): dissolve 50 mg of OPA in 1 mL methanol.

OPA solution (75 mg/L): dilute 75 μ L OPA stock solution (50 mg/mL) to 50 mL with methanol.

OPA reagent: combine 0.8 mL 20 mM borate buffer + 0.2 mL 75 mg/L OPA + 2 μ L 3-mercaptopropionic acid.

FMOC reagent (2.5 mg/mL): dissolve 50 mg of FMOC in 20 mL acetonitrile.

Note: The OPA and FMOC solutions and reagents are sensitive to oxidation and light; therefore, for optimum sensitivity they should be freshly prepared in reagent reservoirs that have been rinsed with methanol.

Preparation of Mobile Phase

Phosphate buffer (50 mM, pH 8.5): dissolve 3.0 g of NaH_2PO_4 in 500 mL water, then adjust to pH 8.5 with 1 M NaOH.

Working Standard Solutions for Calibration

Prepare each amine standard by accurately weighing 25 mg of a standard and diluting to 250 mL in a volumetric flask with water/methanol (1:1, v/v) solution. The concentration of the standard is 100 mg/L (stock standard solution). Prepare four working standard solutions for the calibration with 0.1, 0.5, 1.0, 2.0, and 5.0 μ g/mL concentrations by adding the proper amount of stock standard solution and diluting with a water/methanol (1:1, v/v) solution.

Sample Preparation

Air Sample Extraction

Procedures for the extraction are based on those specified in a publication.²⁶ In this study, particulate matter ($\leq 2.5 \mu\text{m}$) in the atmosphere was collected on a quartz membrane ($\varnothing 90 \text{ mm}$, Waterman, UK) for 48 h at a flow rate of 77.49 L/min using a TSP/PM_{2.5} sampler (Dike Mechanical & Electrical Technology Ltd. Co., Beijing, China) that was located at the top of Building No. 4, Fudan University, Shanghai, China. The compounds of interest were then extracted with 10 mL of water/methanol (1:1, v/v) solution in an ultrasonic bath for 40 min and cooled to room temperature. The extract was filtered through a 0.45 μm membrane (Millex™-LHR) filter prior to derivatization.

Sample Derivatization

The derivatization reactions for primary and secondary aliphatic amines using OPA and FMOC are shown in Figure 1.²⁴ Place the derivatization reagents, borate buffer (400 mM), OPA reagent (0.8 mL 20 mM borate buffer + 0.2 mL 75 mg/L OPA + 2 μL 3-mercaptopropionic acid), FMOC reagent (2.5 mg/mL), and injection dilution buffer (50 mM phosphate buffer, pH 3.0) each in different vials in the autosampler. Automatically execute the derivatization by drawing the derivatization reagents and sample solutions into the sample loop. First, draw reagents and sample solutions into the sample loop in the order of borate buffer (50 μL), OPA reagent (2.5 μL), and sample solution (1 μL); then move the syringe several times to mix the solution. This part of the procedure is for the derivatization of the primary amines. Second, draw FMOC reagent (1 μL) into the sample loop and mix by moving the syringe several times to derivatize the secondary amines.

After derivatization, the derivatized sample solutions are automatically flushed from the sample loop into the analytical flow for amine separations and detection. The whole process is controlled by Chromeleon™ CDS software using the program as shown in Table 1.

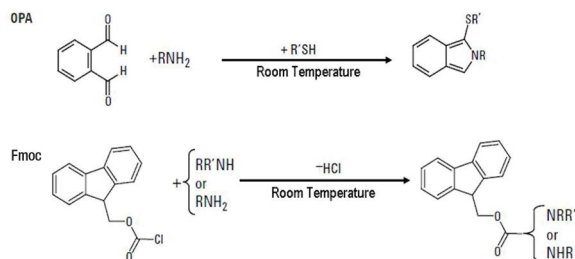


Figure 1. Derivatization reactions of primary and secondary aliphatic amines using OPA and FMOC.

Conditions

Column:	Thermo Scientific Acclaim PolarAdvantage II (PA2), 3 μm Analytical (3.0 \times 150 mm)
Mobile Phase:	A: 50 mM phosphate buffer, pH 8.5 B: CH ₃ CN/CH ₃ OH (1:1, v/v)
Gradient:	B: 0–4 min, 35%; 4–7 min, 35–55%; 7–15 min, 55%
Flow Rate:	0.6 mL/min
Temperature:	40 °C
Detection:	Fluorescence, Ex: 230 nm, Em. 450 nm

Table 1. Program for automatic derivatization

Step	Program	Note
	DrawSpeed = 10.000 [μL/s] DrawDelay = 3000 [ms] DispSpeed = 10.000 [μL/s] DispenseDelay = 0 [ms] WasteSpeed = 10.000 [μL/s] SampleHeight = 2.000 [mm] InjectWash = Both WashVolume = 250.000 [μL] WashSpeed = 25.000 [μL/s] LoopWashFactor = 2.000 PunctureOffset = 0.0 [mm] InjectMode = UserProg ReagentAVial = RA1 ReagentBVial = RB1 ReagentCVial = RC1 ReagentDVial = RD1 SyringeSpeed = GlobalSpeed SampleHeight = GlobalHeight	ReagentAVial: 400 mM borate buffer (pH10.2) ReagentBVial: OPA reagent (0.8 mL 400 mM borate buffer + 0.2 mL 75 mg/L OPA + 2 μL 3-mercaptopropionic acid) ReagentCVial: 2.5 mg/mL FMOC ReagentDVial: 50 mM phosphate buffer (pH 3.0)
1	UdpDraw From = ReagentAVial, Volume = 50.000	Sample vial contains air sample extractions or standard solutions. Derivatize primary amines using OPA reagent.
2	UdpMixWait Duration = 1	
3	UdpDraw From = ReagentBVial, Volume = 2.500	
4	UdpMixWait Duration = 1	
5	UdpMixNeedleWashVolume = 100.000	
6	UdpDraw From = ReagentAVial, Volume = 2.500	
7	UdpDraw From = SampleVial, Volume = 1.000, SampleHeight = 5.000	
8	UdpDraw From = Air, Volume = 6.000	
9	UdpMoveSyringe Load = 6.000 UdpMoveSyringe Unload = 6.000	Repeat Step 9 five times to complete the derivatization.
10	UdpMixWait Duration = 60	Derivatize primary and secondary amines using FMOC reagent.
11	UdpMixNeedleWash Volume = 100.000	
12	UdpDraw From = ReagentCVial, Volume = 1.000,	
13	UdpMixWait Duration = 1	
14	UdpDraw From = Air, Volume = 7.000	Repeat Step 15 eight times to complete the derivatization.
15	UdpMoveSyringe Load = 7.000 UdpMoveSyringe Unload = 7.000	
16	UdpMixNeedleWash Volume = 100.000	Adjust the pH value of the solution in the sample loop to match the mobile phase requirement.
17	UdpDraw From = ReagentDVial, Volume = 14.000	
18	UdpMixWait Duration = 1 UdpDraw From = Air, Volume = 15.000	
20	UdpMoveSyringe Load = 15.000 UdpMoveSyringe Unload = 15.000	Repeat Step 20 five times to thoroughly mix the solution.
21	UdpInjectValve Position = inject	

Results and Discussion

To ensure that the derivatization of primary and secondary amines taking place in the sample loop was completed, the amounts of OPA and FMOC reagents were investigated. Experiments showed that the responses of 2 µg/mL amine standards using OPA (2.5 µL) and FMOC (1 µL) reagents were the same as those using 1/50 amount of OPA and FMOC reagents. This result demonstrated that the amounts of OPA (2.5 µL) and FMOC (1 µL) exceeded the amount required for derivatization of 2 µg/mL amine standards and were enough for the derivatization of common air samples with amine content up to the µg/mL level.

Similar to using a basic buffer mobile phase to separate OPA- and FMOC-derivatized amino acids by reversed-phase HPLC,²⁵ the use of a basic buffer mobile phase for the separation of derivatized aliphatic amines may also produce higher resolution, lower detection limits, and better method reproducibility. Therefore, the Acclaim™ PA2 column that provides enhanced hydrolytic stability from pH 1.5–10 was chosen for use with a basic buffer mobile phase. Figure 2 illustrates good separation of seven amine standards automatically derivatized using OPA (for ethanolamine, methylamine, ethylamine, *n*-propylamine, *n*-butylamine, and *n*-hexylamine) and FMOC (for diethylamine).

Method precision was estimated by making five consecutive injections of a calibration standard with a concentration of 1 mg/L for each amine. The retention time and peak area reproducibilities are summarized in Table 2. Calibration linearity for fluorescence detection of ethanolamine, methylamine, ethylamine, diethylamine, *n*-propylamine, *n*-butylamine, and *n*-hexylamine was investigated by making three consecutive injections of a mixed standard prepared at five different concentrations (i.e., 15 total injections).

The external standard method was used to establish the calibration curve and quantify the analytes in the air samples. Excellent linearity was observed from 0.05 to 5 mg/L when plotting the concentration versus the peak area, and the correlation coefficients were ≥ 0.97 for all. The method detection limits (MDL) of each analyte for fluorescence detection, calculated using signal-to-noise = 3, were all ≤ 20 µg/L.

Figure 3 shows the chromatograms of an air sample as well as a mixture of ethanolamine, methylamine, ethylamine, diethylamine, *n*-propylamine, *n*-butylamine, and *n*-hexylamine standards. No detectable-level aliphatic amines were found except for diethylamine (Peak 4). The calculated concentration of the peak labeled as diethylamine is 20 µg/L, which is similar to the estimated MDL of diethylamine, indicating the presence of diethylamine in the air sample. A small peak with retention time near that of *n*-propylamine (Peak 5) was found in the air sample. However, comparison of the retention times reveals that this peak was from the blank (water). Recoveries for each aliphatic amine standard in the sample ranged from 85 to 95%, demonstrating good accuracy of the HPLC method.

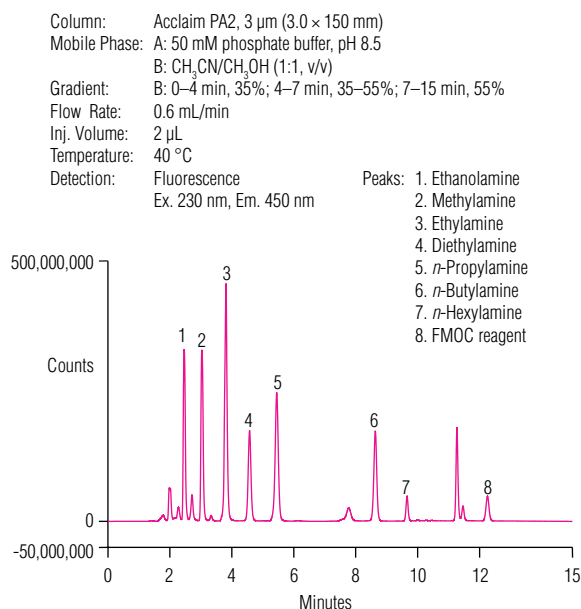


Figure 2. Chromatograms of aliphatic amine standards (1 µg/mL each) automatically derivatized using OPA and FMOC.

Table 2. Reproducibility for peak retention time and area

Analyte	Retention Time RSD	Peak Area (RSD)
Ethanolamine	0.16	2.02
Methylamine	0.26	4.16
Ethylamine	0.44	3.04
Diethylamine	0.38	3.23
<i>n</i> -Propylamine	0.49	0.87
<i>n</i> -Butylamine	0.24	2.27
<i>n</i> -Hexylamine	0.08	0.28

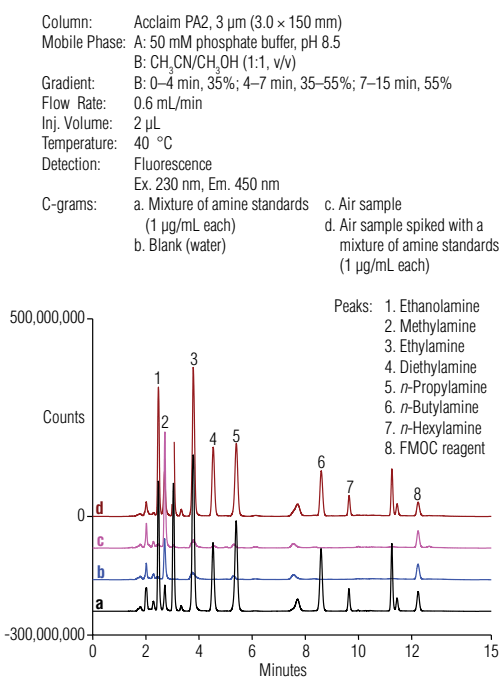


Figure 3. Chromatograms of an air sample and the same sample spiked with a mixture of amine standards.

Conclusion

The work shown here describes an efficient HPLC method using fluorescence detection coupled with automatic precolumn derivatization for the determination of ethanolamine, methylamine, ethylamine, diethylamine, *n*-propylamine, *n*-butylamine, and *n*-hexylamine in air samples. The application of automatic precolumn derivatization reduces the labor required for the determination of aliphatic amines by reversed-phase HPLC

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