Analysis of aliphatic amines in air samples by HPLC with electrochemical detection

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Abstract

A method was developed for the analysis of primary aliphatic amines by high performance liquid chromatography coupled with electrochemical detector. The electrochemical oxidation of aliphatic amines derivatized with 2,5-dihydroxybenzaldehyde was investigated at porous graphite electrodes. The derivatization reactions were performed off-line, before the chromatographic separation. The compounds were separated on a reversed phase column with a methanol–acetonitrile–phosphate buffer and detected setting at an oxidation potential of $+0.5$ V. The influence of the mobile phase buffer concentration and pH on the detector response was also studied. The derivatization was shown to be quantitative and the response linear between 50 and 200 ng/ml. The method is sensitive, selective and could be applicable for the assay of volatile amines in the field of environmental toxicology and also for biological monitoring after occupational exposure. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: HPLC; Electrochemical detection; Aliphatic amines; Derivatization; 2,5-Dihydroxybenzaldehyde; Air

1. Introduction

Several industrial procedures use or cause the formation of aliphatic amines (AA) and the quantitation of these compounds as environmental pollutants is an issue which has been studied [1]. From a toxicological point of view, determination of trace levels of AA in body fluids is of interest for the evaluation of metabolic patterns and for biological monitoring after occupational exposure [2]. Recent works show that chemical compounds of low molecular weight, including volatile AA, cause occupational asthma [3] and the block of voltage-dependent calcium channel in human embryonic kidney cells [4]. Moreover, the structure-toxicity relationship for several aliphatic amines was reported [5,6].

Many analytical methodologies for the quantitation of this kind of amines have been used extensively. A review on works recently published demonstrates that when gas chromatography (GC) [7–10] and high performance liquid chromatography (HPLC) [11–16] methodologies were used, the chemical derivatization were the ideal choice to improve the specificity and sensitivity of the analyses. Other techniques have been described for the determination of AA. Particularly,

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capillary electrophoresis [17,18] and ion chromatography [19–21].

We propose a sensitive and selective method for the analysis of primary aliphatic amines by HPLC coupled with electrochemical detector (ECD). For this purpose we established and optimized a reaction of derivatization of six volatile amines, from the propylamine to octylamine, with 2,5-dihydroxybenzaldehyde (2,5-DBA) to form electroactive Schiff's bases **1–6** measurable by their electrochemical oxidation. In order to optimize the separation and detection several parameters were examined such as oxidation potential, pH and ion strength of mobile phase.

The applicability of this procedure to the assay of AA in air was investigated. Known levels of the described amines in air samples were trapped with commercially available silica gel tubes. The amines were desorbed with an acidic methanolic solution and derivatized prior to HPLC injection.

2. Experimental

2.1. Apparatus

The HPLC apparatus comprised two Model 510 pumps, a Model 712 WISP auto-injector and a Model 490E absorbance detector (Waters Assoc., Milford, MA). An electrochemical detector (Model 5100A Coulochem; ESA, Bedford, MA) which consisted of a control module and an analytical cell (Model 5010) containing two on-line porous graphite coulometric electrodes was used.

The analysis was performed in the oxidative mode and the potential was set at 0.5 V. The ECD sensitivity range and response time were set at 100 nA and 10 s, respectively. Signals from the detectors were converted to chromatographic traces and integrated by an APC IV computer system (NEC, Boxborough, MA) using Maxima 820 software (Waters Assoc., Milford, MA).

^{1}H-NMR spectra were recorded on Varian Unit Inova (220 MHz) instrument in CD_3OD solutions with tetramethylsilane (TMS) as internal standard. Chemical shifts are expressed in ppm (δ).

IR spectra were recorded on a Perkin-Elmer 1600 Fourier transformed spectrometer as KBr

disks. Elemental analysis for C, H, N were obtained on a Carlo Erba 1106 analyzer (Milan, Italy) and agree with theoretical values within $\pm 0.4\%$. Analytical thin layer chromatography (TLC) was performed on Merck 60 F_{254} silica gel plates.

2.2. Chemicals

Chemicals used were obtained from Fluka (Buchs, Switzerland) and were all of the highest purity avalable. All HPLC solvents were purchased from Carlo Erba (Milan, Italy) and were used after filtration through a 0.45 μm filter (Millipore, Bedford, MA). Milli-Q water (Millipore, Bedford, MA) was used.

2.3. Synthesis, isolation and characterization of electroactive compounds

Standards of derivatized amines were prepared as described below and shown in Scheme 1. These were characterized by NMR, IR and elemental analysis. They were then used as external standards to determine percent reaction. The molar ratio between 2,5-DBA and amine was fixed at 2:1. To establish the optimum derivatization time, samples were taken at appropriate time intervals, diluted with acetonitrile filtered and monitored by HPLC-ECD.

2.4. General procedure for amines derivatization

A solution of 2,5-DBA (2.2 mmol) in 2 ml of methanol was added to glacial acetic acid (126 μl,
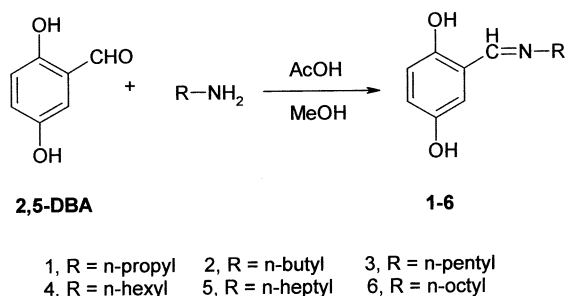
Scheme 1. Reaction of aliphatic amines (AA) with 2,5-DBA to give electroactive derivatives **1–6**

2.2 mmol). Subsequently, suitable pure amine (1.1 mmol) was added. The mixture was stirred at 60 °C for 1 h. The precipitate was removed by filtration, washed with methanol, and dried under vacuum.

2.5. 2-[(propylimino)methyl]benzene-1,4-diol (1)

Yellow crystal, yield 86%; Spectral data: IR: (KBr, cm^{-1}) 3281 (OH), 1639 (–C = N–); ^{1}H-NMR (CD$_3$OD), 8.29 (s, 1H, –CH = N–); 6.89–6.72 (m, 3H, Ar); 3.60–3.57 (m, 2H, –CH = N–CH_2–CH$_2$–CH$_3$); 1.76–1.53 (m, 2H, –CH = N–CH$_2$–CH_2–CH$_3$); 0.88 (t, 3H, –CH = N–CH$_2$–CH$_2$–CH_3).

2.6. 2-[(butylimino)methyl]benzene-1,4-diol (2)

Yellow crystal, yield 85%; Spectral data: IR: (KBr, cm^{-1}) 3278 (OH), 1640 (–C = N–); ^{1}H–NMR (CD$_3$OD), 8.27(s, 1H, –CH = N–); 6.88–6.70 (m, 3H, Ar); 3.61–3.55(m, 2H, –CH = N–CH_2–CH$_2$–CH$_2$–CH$_3$); 1.75–1.53 (m, 2H, –CH = N–CH$_2$–CH_2–CH$_2$–CH$_3$); 1.44–1.27 (m, 4H, –CH = N–CH$_2$–CH$_2$–CH_2–CH$_3$); 0.87 (t, 3H, –CH = N–CH$_2$–CH$_2$–(CH$_2$)$_3$–CH_3).

2.7. 2-[(pentylimino)methyl]benzene-1,4-diol (3)

Yellow crystal, yield 89%; Spectral data: IR: (KBr, cm^{-1}) 3279 (OH), 1639 (–C = N–); ^{1}H-NMR (CD$_3$OD), 8.22 (s, 1H, –CH = N–); 6.87–6.70 (m, 3H, Ar); 3.62–3.54 (m, 2H, –CH = N–CH_2–CH$_2$–(CH$_2$)$_2$–CH$_3$); 1.76–1.51 (m, 2H, –CH = N–CH$_2$–CH_2–(CH$_2$)$_2$–CH$_3$); 1.45–1.26 (m, 4H, –CH = N–CH$_2$–CH$_2$–$(CH_2)_2$–CH$_3$); 0.89 (t, 3H, –CH = N–CH$_2$–CH$_2$–(CH$_2$)$_3$–CH_3).

2.8. 2-[(hexylimino)methyl]benzene-1,4-diol (4)

Yellow crystal, yield 89%; Spectral data: IR: (KBr, cm^{-1}) 3280 (OH), 1639 (–C = N–); ^{1}H-NMR (CD$_3$OD), 8.25 (s, 1H, –CH = N–); 6.88–6.73 (m, 3H, Ar); 3.64–3.53 (m, 2H, –CH = N–CH_2–CH$_2$–(CH$_2$)$_3$–CH$_3$); 1.77–1.50 (m, 2H, –CH = N–CH$_2$–CH_2–(CH$_2$)$_3$–CH$_3$), 1.46–1.23 (m, 6H, –CH = N–CH$_2$–CH$_2$–

$(CH_2)_3$–CH$_3$); 0.89 (t, 3H, –CH = N–CH$_2$–CH$_2$–(CH$_2$)$_3$–CH_3).

2.9. 2-[(heptylimino)methyl]benzene-1,4-diol (5)

Yellow crystal, yield 90%; Spectral data: IR: (KBr, cm^{-1}) 3279 (OH), 1638 (–C = N–); ^{1}H-NMR (CD$_3$OD), 8.22 (s, 1H, –CH = N–); 6.88–6.70 (m, 3H, Ar); 3.65–3.51 (m, 2H, –CH = N–CH_2–CH$_2$–(CH$_2$)$_4$–CH$_3$); 1.76–1.59 (m, 2H, –CH = N–CH$_2$–CH_2–(CH$_2$)$_4$–CH$_3$); 1.49–1.19 (m, 8H, –CH = N–CH$_2$–CH$_2$–$(CH_2)_4$–CH$_3$); 0.89 (t, 3H, –CH = N–CH$_2$–CH$_2$–(CH$_2$)$_4$–CH_3).

2.10. 2-[(octylimino)methyl]benzene-1,4-diol (6)

Yellow crystal, yield 93%; Spectral data: IR: (KBr, cm^{-1}) 3278 (OH), 1635 (–C = N–); ^{1}H-NMR (CD$_3$OD), 8.20 (s, 1H, –CH = N–); 6.87–6.71 (m, 3H, Ar); 3.63–3.49 (m, 2H, –CH = N–CH_2–CH$_2$–(CH$_2$)$_5$–CH$_3$); 1.76–1.58 (m, 2H, –CH = N–CH$_2$–CH_2–(CH$_2$)$_5$–CH$_3$); 1.46–1.17 (m, 10H, –CH = N–CH$_2$–CH$_2$–$(CH_2)_5$–CH$_3$); 0.87 (t, 3H, –CH = N–CH$_2$–CH$_2$–(CH$_2$)$_5$–CH_3).

2.11. Standard solutions

Authentic standards of derivatized amines **1–6** were previously prepared and characterized, so that known concentration of each could be used for accurate quantitation.

Standard solutions of these compounds in the concentration range 50–200 ng/ml were prepared diluting known amounts of acetonitrile stock solution and analyzed by HPLC-ECD. All solutions were stored in the dark at +4 °C.

2.12. Chromatographic conditions

Separations were performed on a 5 μm Prodigy ODS RP-18 column (15 cm × 4.6 mm; Alltech, Milan, Italy) fitted with a guard column (Hypersyl ODS RP-18, 5 μm particles, 4 × 10 mm; Policonsult, Rome, Italy) and eluted, isocratically, with methanol:acetonitrile:buffer phosphate 0.01 M (30/30/40, v/v/v) adjusted to pH 6.5 and contain-

ing 0.2% v/v of triethylamine. The mobile phase was filtered through GS-type filters (0.45 μm Millipore, Bedford, MA) and on-line degassed with a Model ERC-3311 solvent degasser (Erma, Tokyo, Japan). Chromatography was performed at room temperature (22 °C) and at a flow-rate of 1.0 ml/min.

2.13. Air sample collection

Known amounts of amines (200 μmol each), as mixture, were inserted into a closed and warmed system analogous to one reported by the Associazione per l'Unificazione nel Settore dell'Industria Chimica (UNICHIM), official method n. 693 [22]. This system was connected with a silica gel air sampling adsorbent. The air collection was performed according to the standard procedure issued by the UNICHIM method n. 575 [22]. The air in the sample area was sampled at flow rate of 400 ml/min for 4 h using a DuPont Alpha air sample pump (Wilmington, DE).

Double-sealed solid sorbent tubes (ORBO 52) containing silica gel were obtained from SUPELCO (Sigma Aldrich, Milan, Italy).

2.14. Desorption and derivatization

The sample preparations were performed according to the standard procedure described for aliphatic ammines, issued by the National Institute for Occupational Safety and Health (NIOSH) [23] but the sampling tubes were eluted with acetic acid and methanol (1:1). Then, 2,5-DBA was added to the solution and the trapped amines were derivatized with the same technique described previously.

3. Results and discussion

3.1. Optimization of the derivatization procedure

Scheme 1 illustrates the general reaction of selected amines with 2,5-DBA to give the Shiff's base derivatives **1–6**. Experiments were per-
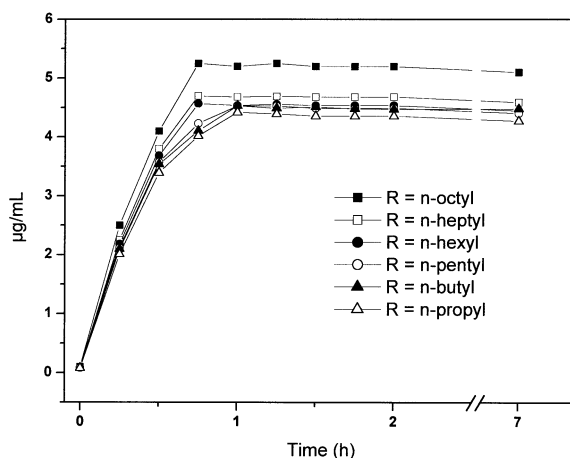

Fig. 1. Time course of the electroactive Schiff 's bases formation (**1–6**) in the optimization of derivatization procedure.

formed to determine optimum derivatization time in order to give maximum conversion of AA to their electroactive derivatives. To this purpose, samples of each reaction were taken at appropriate time intervals (15 min), diluted with acetonitrile, filtered and immediately analysed by HPLC-ECD. Fig. 1 shows the trend of the derivatization procedure which was complete after 60 min with a yield of about 90%. The compounds show the stability in the reaction mixture until 6 h after the optimum.

3.2. Optimization of the electrochemical detection

Several parameters were examined in order to optimize the electrochemical detection of amine derivatives. Electroactive properties of the derivatives **1–6** and 2,5-DBA were examined by their hydrodynamic voltammograms (Fig. 2). Experiments indicated that under the chromatographic conditions reported above, the **1–6** derivatives responded at the ECD oxidation potentials higher than $+0.2$ V. Enhanced signals were obtained as the working potential was increased from $+0.2$ to $+0.5$ V. Fig. 2 indicates that the best potential is $+0.5$ V. With additional applied potential, no increase in peak height occurred and a rise in the background current was observed.

3.3. Chromatographic separations

Chromatographic separations were carried out under isocratic reversed-phase conditions on a Prodigy ODS RP-18 column. The mobil phase consisted of the mixture methanol:acetonitrile: buffer phosphate 0.01 M (30/30/40, v/v/v) at pH 6.5 and at a flow rate of 1 ml/min. The analysis was completed within 36 min. Some parameters were examined in order to optimize the chromatographic separation. The ECD performance was not markedly influenced by the ionic strength of the mobile phase. No significant improvement in the detector response was achieved by increasing concentrations of the phosphate buffer from 0.01 to 0.05 M, which was, consequently, fixed at 0.01 M. The best condition of pH was 6.5. A good robustness was recorded for the pH of the mobile phase because no significant variation in the chromatogram was observed in the 5.0–7.5 pH range.

A typical HPLC-ECD chromatogram of standards was shown in Fig. 3.

3.4. Linearity, detection and quantitation limits

The linearity of response was examined for each derivatized amine, analyzing five solutions in the range 50–200 ng/ml. The coefficients of linear regression of the standard curves were greater
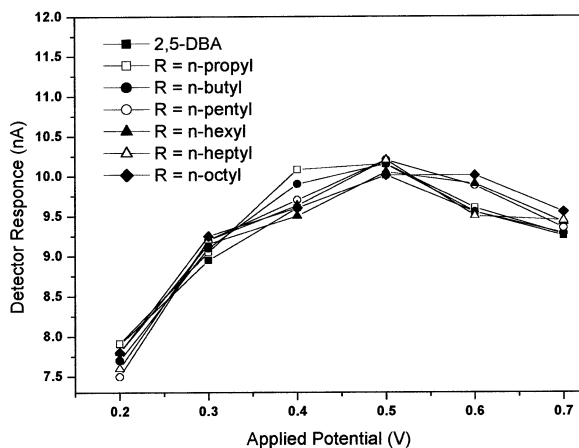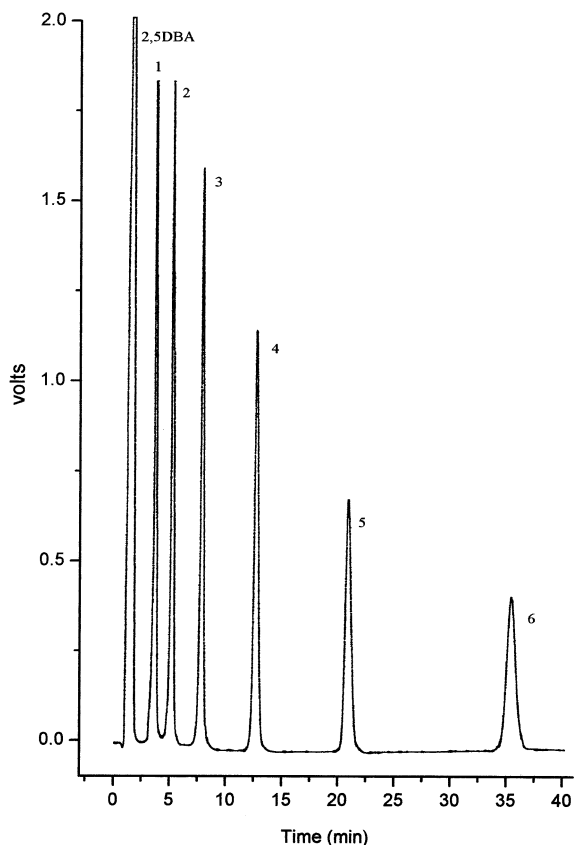
Fig. 3. Typical HPLC-ECD chromatogram of 5 μl (100 ng/ml) of standard mixture of electroactive compounds **1** (3.63 min), **2** (5.08 min), **3** (7.98 min), **4** (12.70 min), **5** (21.20) and **6** (35.93) and 2,5–DBA (1.45 min).

than 0.99. Detection limits (LOD) were determined, from five runs, using progressively lower concentrations of electroactive derivatives for a signal/noise ratio of 3:1 (S/N = 3) with an injected volume of 5 μl. The limit of detection were less than 3 ng/ml for each compound and the limits of quantitation (LOQ) were comprised in the range 18–22 ng/ml.

3.5. Accuracy and precision

The accuracy of the assay was determined by repetitive analysis of air blank samples spiked with 50, 100 and 200 ng/ml of each amines. The accuracy of the assay was determined by comparing the measured concentration to its true value.

Fig. 2. Hydrodynamic voltammograms of electroactive 2,5-DBA and derivatized compounds **1–6**.

Table 1
Accuracy and precision in the analysis of aliphatic amines

True conc. (ng/ml)	Accuracy[a]						RSD[a] (%)					
	Propyl amine	Butyl amine	Pentyl amine	Hexyl amine	Heptyl amine	Octyl amine	Propyl amine	Butyl amine	Pentyl amine	Hexyl amine	Heptyl amine	Octyl amine
50	83.02	80.42	79.20	78.60	78.40	78.20	19.28	21.14	21.97	22.90	23.21	23.63
100	83.10	82.90	82.54	81.49	80.78	79.98	16.24	15.68	16.84	15.46	17.16	17.50
200	82.51	82.05	82.28	82.45	81.23	82.85	16.06	15.60	17.20	16.09	17.38	14.97

[a] $n = 6$.

The reproducibility of the method was evaluated by replicate analysis of the above mentioned air blank sample spiked with a known amount of each amine and was expressed as RSD. The obtained results are reported in Table 1. Recoveries for the studied amines were comprised in the range 78–83%.

3.6. Analysis of air samples

The HPLC-ECD method developed in this study was applied to the assay of AA in air samples. The volatile six amines were completely evaporated inside the system previously reported, were collected, derivatized and underwent HPLC analysis. A typical chromatogram of studied amines in air samples is shown in Fig. 4.
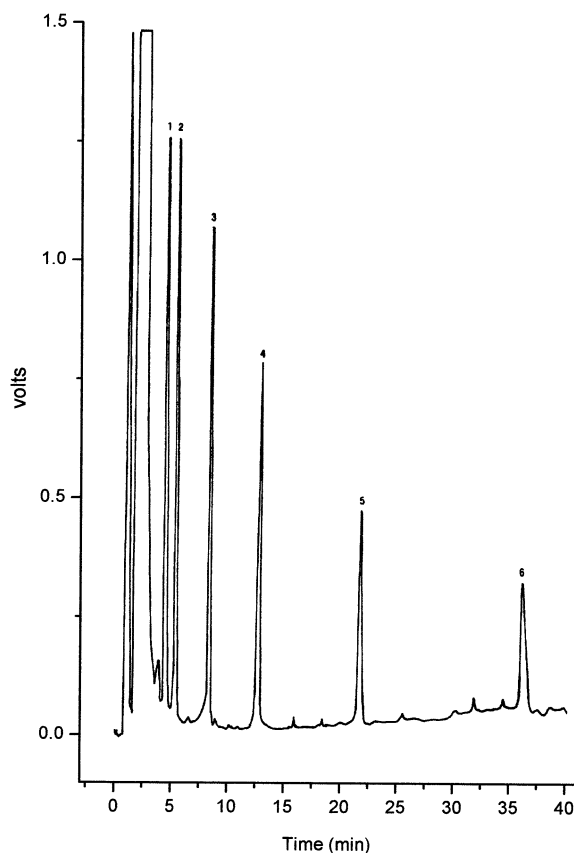

Fig. 4. HPLC-ECD detection of derivatized aliphatic amines in air sample.

For the analysis of the amines we used a molar excess of derivatization agent in order to obtain the maximum yield in electroactive derivatives. Consequently, in the cromatograms of air samples we found a strong peak corresponding to the derivatization reagent 2,5-DBA.

4. Conclusions

The derivatization of AA with 2,5-DBA yield stable sensitive electroactive compounds which are detectable by HPLC-ECD. The sensitivity achieved by this method is sufficiently high to allow the determination of small amounts of aliphatic primary amines. The method was shown to be selective because the applied potential permits the selective oxidation of iminic derivatives. This is useful in determining low levels of these compounds without blank interference due to the limited number of substances which could undergo redox reactions under this condition. The chromatographic analysis with ECD detection indicates that the method is also applicable to air samples. The proposed HPLC method of analysis, due to its selectively and sensitivity for aliphatic primary amines, could be applicable in carrying out controls in the field of the environmental analysis and also for evaluation of small amounts of AA in biological monitoring after occupational exposure.

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