Isolation and Recovery of 2-Aminoethanol, *N*-Methyl-2-Aminoethanol, and *N,N*-Dimethyl-2-Aminoethanol from a Copper Amine Aqueous Matrix and from Amine-Treated Sawdust Using Liquid–Liquid Extraction and Liquid–Solid Extraction Combined with Capillary Gas Chromatography–Ion-Trap Mass Spectrometry

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Abstract

An analytical method for the rapid isolation and recovery of the homologous series of 2-aminoethanols, a class of organic compounds of importance to wood preservative treatment, is successfully developed. The method is applied to an aqueous solution of copper amine (copper[II] hydroxide complexed monoethanolamine) and to copper-amine-treated sawdust. The method incorporates a gas chromatograph–ion-trap mass spectrometer. A discussion of the secondary equilibrium effects involved when ionizable analytes are extracted from an aqueous phase with respect to organic bases is presented. Using 2-propanol as the extractant coupled to a salt-saturated aqueous phase results in recoveries of 63% for 2-aminoethanol, 51% for *N,N*-dimethyl-2-aminoethanol, and 56% for *N*-methyl-2-aminoethanol for a single liquid–liquid extraction. The choice of 2,2,2-trifluoroethanol as an internal standard is found to be quite suitable. A comparison of the precision and accuracy for an external versus an internal mode of instrument calibration demonstrates that the internal standard mode is preferable for this manual injection.

Introduction

The bulk of wood preservatives used for wood protection contain copper, chromium, and arsenic. Migration of heavy metals (mainly arsenic and chromium) from treated wood has received extensive attention. This has resulted in development and commercialization of different copper-based preservative formulations such as copper dimethyldithiocarbamate, copper azole, ammonium copper quaternaries, and other nonmetal-based preservatives. Several new copper-based formulations include an aqueous copper amine solution. The mechanism of copper fixation or resistance to leaching in amine-based copper is not well-understood. Information on retention and binding of copper ion in copper-containing preservatives has been reported (1). In solutions containing copper and zinc, Cooper reported that copper is preferentially absorbed (1). However, in solutions containing copper and organic ligand such as amine, the selective absorption of copper or the amount of amine absorbed in wood is not often reported.

Butcher and Preston studied the toxicity of amine acetates against basidiomycetes and soft rot fungi (2). Although they reported that dodecyldimethylamine acetate was effective against basidiomycetes at a toxic threshold of 1.6–3.2 kg/m^3, no analytical method was reported to determine the amount of amine in wood or in the treating solution. The retention of the amine was determined by weight gain before and after treatment. Considering the potential future of amine-based preservatives, this paper reports on a novel analytical method to determine the amine in treating solution as well as in treated wood using liquid–liquid (LLE) and liquid–solid extraction (LSE) techniques.

Few methods if any can be found in the analytical literature that describe the qualitative and quantitative analyses of samples that contain any of the three 2-aminoethanols (2-AEs). The homologous series begins with 2-AE, and successive substitution for hydrogen by methyl groups leads to the *N*-methyl and *N,N*-dimethyl-2-AE homologues. All three compounds in their neat form are viscous liquids with ammoniac odors. These compounds lack an ultraviolet (UV) absorbing chromophore, and hence 2-AEs are not amenable to analysis of wood-related samples using "common" high-performance liquid chromatograpy (HPLC) –UV techniques. These compounds contain both a polar alcohol and an amino functionality. These polar attributes give rise to secondary chemical equilibrium effects that result in poor extraction

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efficiencies. Traditionally, polar analytes have been converted to semivolatile, less polar derivatives prior to gas chromatographic (GC) analysis (3).

This report describes the successful development of an analytical method without having to resort to chemical derivatization. The method incorporated both LLE and LSE techniques and utilized our recently configured capillary GC–ion-trap mass spectrometer (MS). A commercially available high-resolution capillary GC column was found that exhibited baseline resolution for all three 2-AEs. Mass spectra for each of the three compounds were obtained. The base peak for each 2-AE was then used to develop a selected ion monitoring (SIM) mode of instrument calibration. External standard versus internal standard modes of instrument calibration and verification were established. The precision and accuracy of the two approaches to instrument calibration were compared. LLE and LSE extraction schemes were developed, and recovery percentages for isolating 2-AE, *N*-methyl-2-AE and *N,N*-dimethyl-2-AE were measured. The method was applied to amine-treated sawdust and to copper amine aqueous solutions.

Experimental

Instrumentation

An Autosystem GC (Perkin-Elmer, Norwalk, CT) has been modified by interfacing it to a Series 800 Ion Trap Mass Spectrometer (ITD) (Finnigan, San Jose, CA). The interface was accomplished by replacing the flame-ionization detector with the transfer line of the ITD; a schematic diagram is shown in Figure 1. There are two ways that the GC can be interfaced with the ITD. One approach is termed an *open split interface*. A second approach is termed a *direct coupling interface* whereby the GC capillary column is brought through the transfer line after the flow restrictor has been removed and the column has been extended into the ion trap manifold. This direct coupling leads to an increase in ITD sensitivity and is considered a more appropriate interface for organic compounds with higher molecular weights and for compounds that are more polar (4). An IBM PC XT via the Ion Trap Data System (ITDS) software (Finnigan) controlled and monitored the system temperatures and ITD scanning parameters. It also acquired data and displayed it on the video monitor. Calibration was accomplished using the ITDS software Quantitation Program features, which included SIM mode.

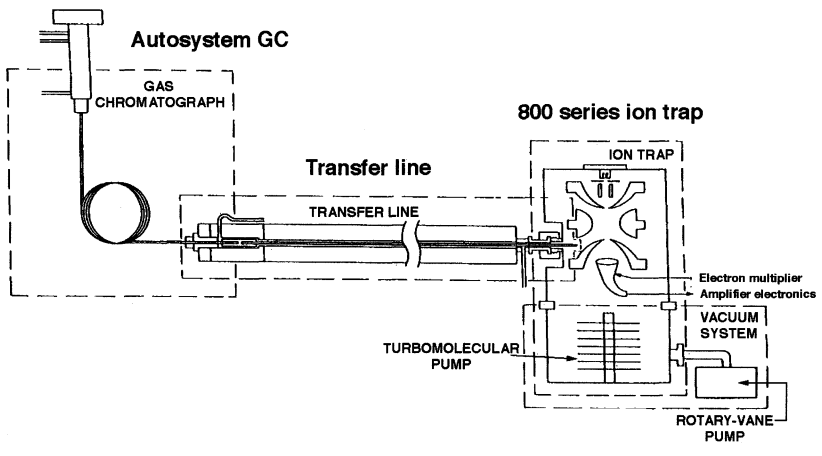


Figure 1. Schematic diagram for the capillary GC–MS (ITD) configuration. An Autosystem (Perkin-Elmer) GC was interfaced to a Series 800 (Finnigan) ion-trap mass spectrometer (ITD).

Total ion chromatograms and accompanying mass spectra were transferred from the 5½-inch floppy to a 3½-inch diskette. The ITDS software was loaded on a 486 PC (Gateway 2000, North Sioux City, SD) along with software designed to "capture" the screen. This technique enabled us to take advantage of more contemporary computer technology and therefore produce a higher quality graphics display.

A 30-m × 0.25-mm DB-624 (J&W Scientific, Folsom, CA) capillary column was installed into the modified GC–MS (ITD) described above using the direct coupling interface. The temperature program that retained all three 2-AEs and optimized chromatographic resolution among all three 2-AEs started at 50°C for 3 min and was then increased to 100°C at a rate of 10°C/min. At approximately 80°C, 2-AEs began to elute from the column.

A splitless mode of injection with a closed split vent time interval of 0.5 min combined with SIM exhibited the most sensitivity for all three analytes of interest.

Reagents

The 2-AE, *N*-methyl-2-AE, and *N,N*-dimethyl-2-AE were obtained from Aldrich Chemical (St. Louis, MO) and were used without further purification. All solvents were ACS-grade or higher and were used without further purification. ACS-reagent-grade sodium chloride was obtained from Columbus Chemical Industries (Columbus, WI).

Apparatus

A model 2200 bath sonicator (Branson Ultrasonics, Danbury, CT) was used to perform the liquid–solid leaching of the sawdust samples. Glassware used to perform LLE of the spiked water and aqueous copper amine samples consisted of 20-mL glass vials or 125-mL separatory funnels.

Sawdust sample preparation

A 0.5-g portion of the sawdust was placed in a 150-mL beaker. A 10-mL volume of methanol (MeOH) was added, and the mixture bath was sonicated for 5 min. The mixture was allowed to settle for at least 10 min. A quantitative transfer of the supernatant was made by decanting the clear liquid into a 10-mL volumetric flask that already contained 100 µL of 11,000-ppm 2,2,2-trifluoroethanol, which was used as an internal standard. The volume was adjusted to the calibration mark with the supernatant, and 1 µL of this extract was injected into the GC–MS.

Sample preparation for an aqueous solution containing a Cu-2-AE complex

Enough sodium sulfate was added to a 4-mL aliquot of the deep-blue liquid to saturate the solution. Then 2 mL of isopropanol was added and shaken in a glass vial. A 1-µL portion of the top layer was injected into the GC–MS.

Results and Discussion

Observations from GC–MS

The principles of the ITD have been introduced in a number of texts (5). Figure 2 shows a total ion chromatogram for injection of a methanolic mixture containing all three 2-AEs at the concentrations indicated. A delay in turning on the current to the filament is a routine procedure that we use to extend filament life. This results in the absence of a baseline for the first 300 scans, as is evident in Figure 3. The elution order is given in the figure and did not correlate with either polarity or boiling point. The DB-624 is a chemically bonded derivatized polysiloxane-coated fused-silica capillary column (proprietary) specifically designed for the analysis of volatile priority pollutants from J&W Scientific. We could not reproduce the high degree of chromatographic resolution we have enjoyed on the DB-624 among all three 2-AEs on either a DB-5 or cyanopropyl column or via direct aqueous injection on a Carbopack (Supelco, BelleFonte, PA) packed column.

Each of the three compounds yielded unique mass spectra based on the injection of individual 2-AEs. Figure 3 is an ITD mass spectrum for 2-AE. The most abundant ions were found with a mass-to-charge ratio of *m/z* 62, even though their molecular weight is 61 and they are referred to as M + 1 ions. Figure 4 shows an ITD mass spectrum obtained from our instrument of *N,N*-dimethyl-2-AE whose molecular weight is 89 and shows a large abundance at *m/z* 90 (M + 1). The abundance at *m/z* 72 resulted from loss of a water molecule and the abundance at *m/z* 58 arose from the formation of the stable $(CH_3)_2N{=}CH_2^+$ ion. Figure 5 shows an ITD mass spectrum of *N*-methyl-2-AE whose molecular weight is 75 and shows again an abundant M + 1 ion at *m/z* 76. The mass range was begun at 50 amu due to the presence of oxygen, nitrogen, and carbon dioxide impurities in our carrier gas. Given this mass spectral information from running the pure 2-AEs as if they were reference standards, we proceeded to use SIM and used the unique M + 1 ion for each of the three 2-AEs in subsequent studies.

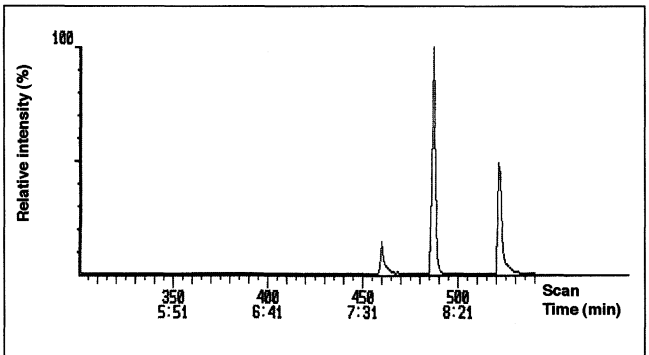


Figure 2. Capillary GC–MS (ITD) total ion chromatogram for the injection of 1 µL of a methanolic reference standard containing (in order of elution) 104 ppm 2-AE, 94 ppm *N,N*-dimethyl-2-AE, and 132 ppm *N*-methyl-2-AE. Refer to the Experimental section for conditions.

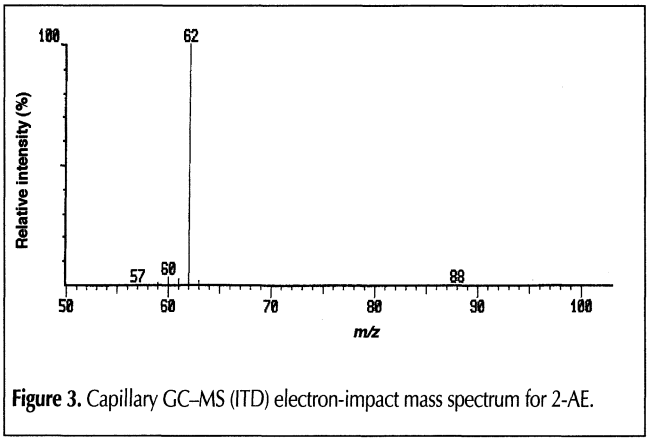


Figure 3. Capillary GC–MS (ITD) electron-impact mass spectrum for 2-AE.

Basis for isolating 2-AEs from aqueous samples via LLE

Nomenclature, molecular structure, and acid dissociation constants for the three 2-AEs that are the focus of study in this paper are shown in Table I. The presence of an amino group gives rise to secondary chemical equilibria. It is therefore possible for a significant dependence of sample pH on 2-AE recovery to occur. A derivation of the dependence of the distribution ratio (*D*) on pH is developed below. For 2-AE, *N*-methyl-2-AE, and *N,N*-dimethyl-2-AE dissolved in water, the following base dissociation equilibria were established using a generalized molecular formula:

$$R_2N\text{-}CH_2\text{-}CH_2\text{-}OH + H_2O \overset{K_b}{\rightleftharpoons} [R_2NH\text{-}CH_2\text{-}CH_2\text{-}OH]^+ + OH^-$$

$$K_b = \frac{[R_2NH\text{-}CH_2\text{-}CH_2\text{-}OH]^+ [OH^-]}{[R_2N\text{-}CH_2\text{-}CH_2\text{-}OH]} \qquad \text{Eq 1}$$

The distribution ratio (*D*) can be defined as the ratio that

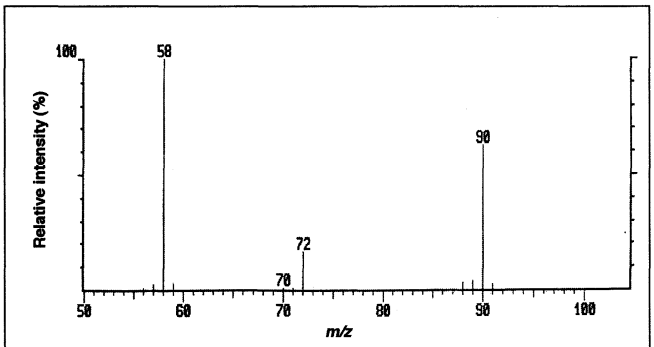


Figure 4. Capillary GC–MS (ITD) electron-impact mass spectrum for *N,N*-dimethyl-2-AE.

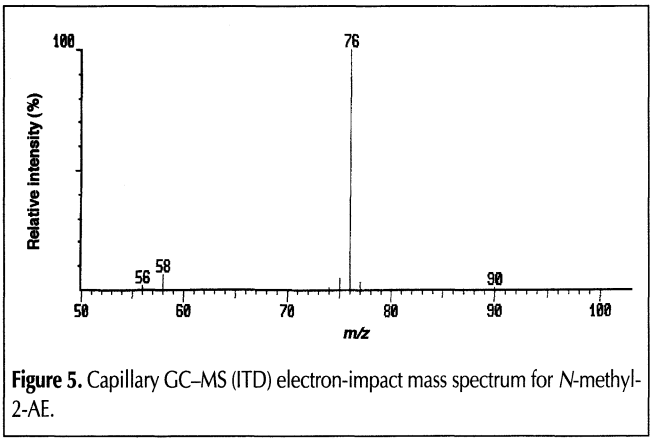


Figure 5. Capillary GC–MS (ITD) electron-impact mass spectrum for *N*-methyl-2-AE.

accounts for all forms of 2-AEs distributed between an organic phase and an aqueous phase. Assuming that only the neutral form exists in the organic phase, we can write an expression for D in the following manner (6):

$$D = \frac{\text{total analyte in organic phase}}{\text{total analyte in aqueous phase}}$$

$$D = \frac{[R_2N\text{-}CH_2\text{-}CH_2\text{-}OH]_{organic}}{[R_2N\text{-}CH_2\text{-}CH_2\text{-}OH]_{aqueous} + \{[R_2NH\text{-}CH_2\text{-}CH_2\text{-}OH]^+\}_{aqueous}} \quad \text{Eq 2}$$

The molecular partitioning distribution constant is a thermodynamic term that describes the extent to which neutral molecules redistribute themselves between an aqueous polar phase and a liquid nonpolar phase at room temperature. It is defined as follows:

$$K_D = \frac{[R_2N\text{-}CH_2\text{-}CH_2\text{-}OH]_{organic}}{[R_2N\text{-}CH_2\text{-}CH_2\text{-}OH]_{aqueous}} \quad \text{Eq 3}$$

Recall that the sum of the pK_a and the pK_b equals the pK_w and that the product of the hydronium and hydroxide ion concentrations equals the K_w. Upon rearranging and simplifying, an expression that relates the distribution ratio to the pH emerges:

$$D = \frac{K_D}{\{1 + [H^+]/K_a\}} \quad \text{Eq 4}$$

Equation 4 shows that a decrease in $[H^+]$ that corresponds to an increase in pH should eliminate the secondary equilibrium. It is easily seen that, in the case of the homologous series of methyl-substituted 2-AEs, D approaches K_D in the limit as the $[H^+]$ became one or two orders of magnitude lower than the corresponding K_a value for each compound.

Recall that the percent recovery for LLE is defined in terms of D and the ratio of the organic phase volume ($V_{organic}$) to the aqueous phase volume ($V_{aqueous}$) as follows:

$$E = \frac{[D]\,[V_{organic}/V_{aqueous}]}{1 + [D]\,[V_{organic}/V_{aqueous}]} \quad \text{Eq 5}$$

Also consider that $100E$ is the recovery percentage. Equation 4 suggests, with respect to performing only a single extraction, that to maximize the recovery percentage for a given analyte requires a change in extraction solvents, which might increase the value of D.

Preliminary LLE and LSE method development results

Our first attempt to extract an aqueous matrix resulted in a predictable outcome. A methanolic reference standard (200 µL) containing 10,000 ppm of each 2-AE was added to 50 mL of distilled, deionized water (DDI). The pH was adjusted to 11 to suppress the proton-accepting nature of the primary amino functional group on 2-AE. Two approaches for LLE could lead to increased partitioning of all three 2-AEs. The first approach would be to investigate a more polar extracting solvent, which would change the magnitude of D, whereas the second approach, increasing the volume of the organic extractant, would increase the $V_{organic}/V_{aqueous}$ ratio, according to Equation 5.

Table I. Nomenclature, Molecular Structure, and Acid Dissociation Constants for the 2-AEs Studied in this Paper

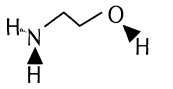
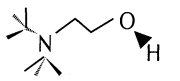
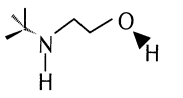
Compound	Structure	pK_a
2-AE		9.50
N,N-dimethyl-2-AE		9.26
N-methyl-2-AE		9.88

Table II. Selected Solvents Used in the Study of Isolation and Recovery of 2-AEs*

Solvent	Polarity† (P')	Density‡ (µg/mL)	Solubility in water (%)
Hexane	0.1	0.6594	0.001
Iso-octane	0.1	0.6919	0.0002
Methyl-*t*-butyl ether	2.5	0.741	4.8
Methylene chloride	3.1	1.326	1.6
2-Propanol	3.9	0.7854	100
EtOAc	4.4	0.9006	8.7

* Taken from reference 8.
† Polarity index (P') is a relative measure of the degree of interaction between the solvent and various polar test solutes. P' increases with increasing solvent polarity.
‡ At 20°C.

Table III. Summary of Preliminary LLE and LSE Partitioning Studies for All Three 2-AEs via Capillary GC–MS (ITD)

			Recovery (y/n)			
Solvent	Matrix	Vessel	2-AE	*N,N*-dimethyl-2-AE	*N*-dimethyl-2-AE	
$MeCl_2$	DDI	open	no	yes	no	MeOH
$MeCl_2$	cellulose filter paper	open	yes	no	no	MeOH
$MeCl_2$–Ac_2O (1:1)	cellulose filter paper	open	yes	no	yes	MeOH
$MeCl_2$	fiber filter paper	open	no	yes	yes	DDI
$MeCl_2$	fiber filter paper	open	yes	no	yes	EtOAc
$MeCl_2$	cellulose filter paper	semiclosed	yes	yes	yes	none
$MeCl_2$–Ac_2O (1:1)	cellulose filter paper	open	yes	yes	yes	none

Table IV. A Comparison of the External and Internal Standard Modes of Capillary GC–MS (ITD) –SIM Calibration for 2-AE*

Stastical parameter	External standard	Internal standard
Correlation coefficient over *n* calibration points	0.981 (*n* = 4)	0.978 (*n* = 5)
ICV (ppm) expected	270	270
ICV (ppm) mean over *n* replicate injections	244.5 (*n* = 6)	260.1 (*n* = 7)
Confidence interval at 95% significance† (ppm)	20.8	5.0
Coefficient of variation‡	8.1 (*n* = 6)	2.1 (*n* = 7)
Relative error§	9.4	3.7

* GC conditions: column, 30 m x 0.25 mm DB-624 (J&W Scientific); temperature program, 50°C (held for 4 min) increased to 100°C at 10°C/min (held for 1 min); carrier gas, helium (1 cc/min); injection, splitless with vent closed for the first 0.5 min. 2,2,2-Trifluoroethanol was used as the internal standard whose retention time is 4.05 min.

† Confidence interval at 95% significance means that 95 out of every 100 subsequent injections of the ICV should fall within the confidence interval about the mean value in parts per million.

‡ Coefficient of variation was calculated from the ratio of the standard deviation to the mean. Coefficient of variation = (standard deviation among *n* replicate injections of the ICV/mean concentration [ppm]) x 100.

§ Relative error was calculated from the ratio of the difference between the measured ppm and the expected ppm to the expected ppm according to: Relative error = $[|X_{measured} - X_{expected}|/X_{expected}] \times 100$.

Table V. A Comparison of the External and Internal Standard Modes of Capillary GC–MS (ITD) –SIM Calibration for *N,N*-Dimethyl-2-AE*

Statistical parameter	External standard	Internal standard
Correlation coefficient over *n* calibration points	0.986 (*n* = 4)	0.999 (*n* = 5)
ICV (ppm) expected	235	235
ICV (ppm) mean over *n* replicate injections	229.5 (*n* = 6)	235 (*n* = 7)
Confidence interval at 95% significance (ppm)	23.2	9.6
Coefficient of variation	9.6 (*n* = 6)	4.4 (*n* = 7)
Relative error	2.3	0.04

* Refer to Table IV for experimental details.

Table VI. Comparison of the External and Internal Standard Modes of Capillary GC–MS (ITD) –SIM Calibration for N-Methyl-2-AE*

Stastical parameter	External standard	Internal standard
Correlation coefficient over *n* calibration	0.985 (*n* = 4)	0.994 (*n* = 5) points
ICV (ppm) expected	330	330
ICV (ppm) mean over *n* replicate injections	306.3 (*n* = 6)	316.6 (*n* = 7)
Confidence interval at 95% significance	39.0	18.1 (ppm)
Coefficient of variation	12.1 (*n* = 6)	6.2 (*n* = 7)
Relative error	7.2	4.1

* Refer to Table IV for experimental details.

The aqueous matrix was then extracted three successive times using 10 mL of methylene chloride each time. We observed recovery of only the *N,N*-dimethyl-2-AE. This was not surprising considering that the *N,N*-dimethyl-2-AE is more hydrophobic due to methyl substitution in contrast to both 2-AE and *N*-methyl-2-AE. This may explain the significant differences in K_D values among all three 2-AEs studied.

To simulate a wood sample that might be saturated with one or more 2-AEs, standard cellulosic and glass fiber filter paper was used in an open vessel. The paper was spiked initially with the methanolic reference standard containing 10,000 ppm of each 2-AE, and the paper was allowed to dry overnight. Upon extracting with methylene chloride, we observed a high recovery of 2-AE only. However, upon extracting with a 50:50 mixture of methylene chloride and acetone, we recovered 2-AE and *N*-methyl-2-AE but not *N,N*-dimethyl-2-AE.

Glass fiber filters were spiked in the next series of preliminary evaluation of 2-AE recovery. Table II lists the solvents used in this paper along with their relevant properties. The three 2-AEs were dissolved in DDI and in ethyl acetate. Again 200 µL of an aqueous or ethyl acetate reference standard containing 10,000 ppm of each 2-AE was impregnated onto the glass fiber filter. The filters were allowed to dry overnight and were subsequently extracted with methylene chloride. We were surprised to find that when DDI was the solvent used in the spiking reference standard, *N,N*-dimethyl-2-AE exhibited a high recovery, whereas *N*-methyl-2-AE yielded a low recovery, and 2-AE gave no recovery. When ethyl acetate was the solvent used in the spiking reference standard, *N,N*-dimethyl-2-AE was not recovered at all, whereas *N*-methyl-2-AE and 2-AE gave high recoveries. Several days later, injection of the 10,000-ppm spiking reference standard showed no 2-AE to be present. Esters are known to react with ammonia to form amides, and considering that the 10,000 ppm concentration of 2-AE in the presence of a large excess of ethyl acetate may have led to the formation of 2-hydroxyethyl acetamide, we did not pursue this any further.

Next we eliminated the solvent from the spiking reference and impregnated the neat form of all three 2-AEs onto cellulose type filter paper in an attempt to simulate a wood matrix. This impregnation was carried out in a semiclosed vessel. Upon extracting into 10 mL methylene chloride, all three 2-AEs were recovered. To the same filter paper, equal amounts of all three 2-AEs were impregnated again. The paper was allowed to stand exposed to air, and an air purge was then introduced. The paper was then extracted into 10 mL of a 1:1 mix-

ture of methylene chloride and acetone. All three 2-AEs were recovered.

The results from this series of preliminary recovery studies are summarized in Table III. It appears that several factors significantly influenced the extent to which all three 2-AEs could be isolated and recovered from both an aqueous and a solid sample matrix using LLE and LSE techniques. The more hydrophobic *N,N*-dimethyl-2-AE would be expected to have a large K_D value for the partitioning from an aqueous phase to a nonpolar phase. Our results, as shown in the first row of Table III, confirmed the theoretical concept. However, once the aqueous phase was eliminated, the two more hydrophobic 2-AEs that were adsorbed to the cellulose surface evaporated much more rapidly than 2-AE itself. This explains the complete loss of *N,N*-dimethyl-2-AE and *N*-methyl-2-AE as is shown in the second row of Table III. Evaporative loss of *N,N*-dimethyl-2-AE explains the observations shown in the third row of Table III. Using a glass fiber filter instead of cellulosic filters as the substrate led to further differences in recovery among the three 2-AEs, as is evident in rows 4 and 5 of Table III. Comparing results from rows 2 and 6 of Table III shows

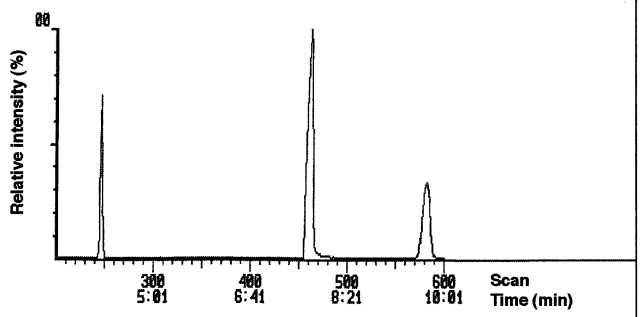


Figure 6. Capillary GC–MS (ITD) total ion chromatogram for the injection of 1 µL of a methanolic extract from a sample of wood sawdust which had been previously treated with 2-AE. The sample was stored in a closed glass jar prior to sample preparation. The sample was extracted using the bath sonication–LSE technique. The peak at approximately 200 scans is due to the internal standard, 2,2,2-trifiuoroethanol, and the peak at approximately 460 scans is due to 2-AE. The peak at approximately 580 scans in the chromatogram is unidentified.

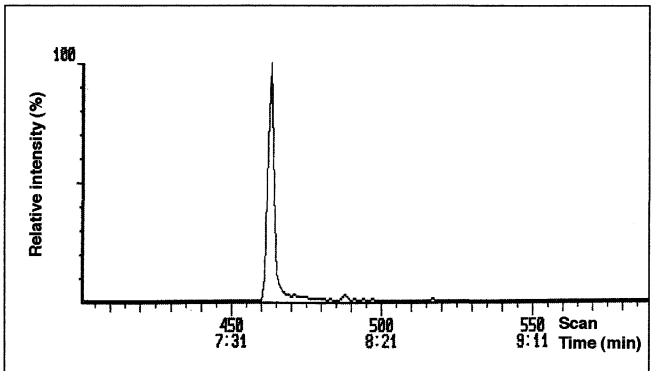


Figure 7. Capillary GC–MS (ITD) total ion chromatogram for the injection of 1 µL of a 2-propanol extract from a sample consisting of an aqueous solution containing a copper (II) hydroxide complexed with 2-AE complex. The sample was stored in a glass test tube prior to sample preparation. The sample was saturated with NaCl then extracted with 2-propanol. The peak at approximately 460 scans corresponds to 2-AE.

that the use of a semiclosed vessel prevented the more hydrophobic compounds from evaporating. Comparing rows 3 and 7 of Table III shows a dependence on the solvent used to prepare the spiking solution. Perhaps the neat form of these three compounds, all of which are liquids at room temperature, enabled the molecules to more effectively penetrate the cellulose surface and hence not become as available for mass transport from surface to air in the absence of methanol.

Instrument calibration study

The excellent resolution that was obtained for all three 2-AEs combined with the lack of an autosampler suggested that we evaluate the precision and accuracy of the analytical method being developed. This was accomplished by first establishing a multipoint calibration over all three analytes using the external mode of instrument calibration and using the SIM mode with *m/z* 62 selected for 2-AE, *m/z* 90 for *N,N*-dimethyl-2-AE, and *m/z* 76 for *N*-methyl-2-AE. We then repeated the calibration using an internal standard mode of instrument calibration and SIM. An initial calibration verification reference standard (ICV) was then injected a replicate number of times to establish both precision and accuracy. The ICV is an additional working standard, generally taken from near the mid-range of the calibration. Use of an ICV is considered good quality control in trace environmental analysis according to EPA methodology (7). The ICV is interpreted as if it were an unknown, and the instrument response is interpolated using the calibration plot. If the ICV is injected a replicate number of times, two indicators of precision can be obtained: the confidence interval (CI) at 95% significance and the coefficient of variation. How close the mean concentration for the ICV is to that expected serves to specify the accuracy for the calibration plot as measured by the relative error. An internal standard that was chemically similar to the 2-AEs was selected, and fortunately its retention time did not interfere with the retention times of the three 2-AEs.

The results of this comparative study for all three 2-AEs are shown in Tables IV, V, and VI. The internal standard mode of instrument calibration gave significantly improved precision, as shown by the lower coefficient of variations for all three 2-AEs as well as improved accuracy shown by the lower relative error. The two more hydrophobic compounds, *N,N*-dimethyl2-AE and *N*-methyl-2-AE, exhibited calibration plots which were more closely

Table VII. Analytical Results for the Quantitative Determination of 2-AE in a Liquid Sample and Several Samples of Sawdust

Unknown sample-identifier*	Concentration found
A	5,000 µg/g
B	70,000 µg/g
A1	70,000 µg/g
B1	5,200 µg/g
Control	4,800 µg/g
Liquid, labeled 8% EA (deep blue)†	20,300 µg/mL

* Obtained from the Department of Forestry at Michigan State University.
† Consists of copper (II) hydroxide dissolved in water and complexed with 2-AE at an expected 2-AE concentration of 8% (80,000 ppm).

fitted by a linear least squares regression when compared to 2-AE. The somewhat tailed peak shape of the polar 2-AE which gets more pronounced as the 2-AE concentration is increased may help to explain this difference among all three 2-AEs.

Recovery from immiscible 2-propanol–aqueous salt-saturated system

One of the ways to increase D and in turn E (refer to Equations 4 and 5) and therefore lead to higher recovery percentages is to find a more polar solvent to use to extract the 2-AEs. However, most polar solvents such as lower molecular weight alcohols are completely miscible with water. It has been recently suggested that a two-phase system can be created that consists of a salt-saturated aqueous phase in contact with 2-propanol (G. Matthijs. Private communication, 1996). This yields two immiscible liquid phases. The partitioning of polar 2-AEs from a salt-saturated aqueous phase to a less polar 2-propanol phase is expected to lead to increased recovery percentages. A salt-saturated aqueous solution was spiked with the 2-AEs resulting in recovery percentages of 63% for 2-AE, 51% for *N*,*N*-dimethyl-2-AE, and 56% for *N*-methyl-2-AE. This was a preliminary recovery percentage study that demonstrated good recovery from a single extraction. It is expected that successive LLEs coupled with a variation in $V_{organic}/V_{aqueous}$ (refer to Equation 5) would lead to an increase in recovery percentage. An exhaustive study of the recovery percentage of 2-AEs was not pursued.

Application to real samples

The LLE technique using 2-propanol and saturating an aqueous sample with salt and the bath sonication LSE technique were applied to a series of 2-AE-treated samples. The samples were expected to contain only 2-AE. 2-AE was easily isolated and recovered using the LLE–salt-saturated aqueous / 2-propanol technique. 2-AE was also easily isolated and recovered using the bath sonication / LSE technique from five sawdust samples. Table VII lists the analytical results obtained. Figure 6 shows a total ion chromatogram from a methanolic extract of a 2-AE-treated sawdust sample. Figure 7 is a typical total ion chromatogram of the 2-propanol extract which was diluted 1:10 with 2-propanol from the liquid sample. This sample was expected to contain 8% of 2-AE in an aqueous solution containing copper (II) hydroxide.

Conclusion

A novel analytical method has been developed that isolates and recovers the polar *N*-methyl-substituted 2-AEs from both an aqueous matrix and treated sawdust. This method should be applicable to other polar molecules that also exhibit secondary equilibrium effects.

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