RESIDUES AND TRACE ELEMENTS

Stability of Organic Pollutants During Microwave-Assisted Extraction from Solid Matrixes

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A stability study was conducted for 95 semivolatile organics listed in U.S. Environmental Protection Agency Method 8250 (this number includes 6 surrogate compounds). These compounds were spiked into solvent only [hexane–acetone (1 + 1), methylene chloride–acetone (1 + 1), toluene–methanol (10 + 1), and methyl *tert*-butyl ether], solvent/dry soil suspensions, and solvent/wet soil suspensions [20% water (w/w)] and heated with microwave energy in closed vessels at 50° or 145°C for 5 or 20 min. For comparison and to determine nitrogen blowdown losses, spiked solvent samples that had not been exposed to microwave energy were concentrated by the blowdown technique and analyzed for each of the spiked compounds. Hexane–acetone (1 + 1) seems to be the best for the compounds and matrixes investigated, with recoveries > 80%, except for basic compounds and benzoic acid in the solvent/dry soil suspension experiments. Increasing extraction time from 5 to 20 min did not increase recoveries; in fact, recoveries of neutral compounds decreased slightly at the longer extraction time. Increasing the temperature from 50° to 145°C decreased recoveries of basic compounds by about 10%. Recoveries of basic compounds, of benzoic acid (and probably other organic acids as well), and at least to some extent of phenolic compounds from the solvent/wet soil suspensions were higher than those for solvent/dry soil suspensions.

In earlier studies (1, 2) we reported on the extractability under microwave-assisted extraction (MAE) conditions of 95 compounds listed in U.S. Environmental Protection Agency (EPA) Method 8250, 45 organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) listed in EPA Method 8081, and 47 organophosphorus pesticides listed in EPA Method 8141 from freshly spiked soil samples; spiked soil samples that had been aged for 24 h, 14 days, or

21 days; and a few standard reference materials. For these extractions, we used hexane–acetone (1 + 1) at 115°C for 10 min.

Solvents other than hexane–acetone have been used for more polar compounds (3); however, none of the solvents recommended by EPA in Methods 3540 and 3550 (e.g., methylene chloride–acetone and toluene–methanol) has been evaluated thoroughly for use under MAE conditions. Furthermore, degradation or conversion of compounds that may occur when using microwave energy to heat the solvent/soil suspension has not been investigated. Possible ways in which compound degradation may occur include exposure to temperature and pressure inside the microwave extraction vessel, interaction with other analytes or with solvent under these conditions, and catalysis by the matrix. To determine whether degradation under MAE conditions presents a problem, a stability study was conducted for 95 semivolatile organics listed in EPA Method 8250 (this number includes 6 surrogate compounds). These compounds were spiked into solvent only (hexane–acetone, 1 + 1; methylene chloride–acetone, 1 + 1; toluene–methanol, 10 + 1; and methyl *tert*-butyl ether), solvent/dry soil suspensions, and solvent/wet soil suspensions (20% water, w/w) and heated in closed vessels with microwave energy at 2 temperatures (50° or 145°C) for 5 or 20 min. For comparison and to determine nitrogen blowdown losses, spiked solvent samples that had not been exposed to microwave energy were concentrated by the blowdown technique and analyzed for each of the spiked compounds. Recoveries reported here have been corrected for blowdown losses.

Experimental

Standards

Analytical reference standards of the 95 compounds were purchased from Absolute Standards, Inc. (Camden, CT) as 8 composite solutions in methylene chloride (mix 1 consisting of 14 ethers, phthalates, and nitrosamines; mix 2 of 14 compounds, mostly of chlorinated benzenes, nitrobenzene, and nitrotoluenes; mix 4

of 3 phenols and benzoic acid; mix 5 of various anilines, dibenzofuran, benzyl alcohol, and 2-methylnaphthalene; mix 8 of 13 phenols; mix 9 of 8 miscellaneous compounds; mix 10 of ethyl methanesulfonate and methyl methanesulfonate; and mix 11 of 11 nitrogen-containing compounds), one composite solution in methanol (mix 6 consisting of benzidine and 3,3'-dichlorobenzidine), and one composite solution in methylene chloride–benzene (1 + 1) consisting of 17 polynuclear aromatic hydrocarbons. In these mixtures, the concentrations of each compound was 2 mg/mL. Dibenzo(a,j)acridine was purchased from Chem Service (West Chester, PA) and 1,2-diphenylhydrazine from Aldrich Chemical (Milwaukee, WI). The 6 surrogate compounds listed in Table 1 were purchased from Absolute Standards as 2 composite solutions: the acid surrogate standard contained 2-fluorophenol, phenol-d_5, and 2,4,6-tribromophenol at 2 mg/mL in methanol, and the base/neutral surrogate standard contained 2-fluorobiphenyl, terphenyl-d_{14}, and nitrobenzene-d_5 at 1 mg/mL in methylene chloride. Six internal standards (1,4-dichlorobenzene-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}) were purchased from Supelco, Inc. (Bellefonte, PA) as a composite solution at 2 mg/mL in methylene chloride; their purities were stated to be 99%. An intermediate stock solution of all target compounds at 125 μg/mL (except for 2 fluorobiphenyl, nitrobenzene-d_5, and terphenyl-d_{14} at 62.5 μg/mL) was prepared by combining calculated amounts of the various composite solutions and diluting to volume with methylene chloride. Calibration standards at 5, 10, 15, and 50 μg/mL were prepared by serial dilution with methylene chloride of the 125 μg/mL composite solution. The 6 internal standards were spiked into every calibration standard and sample extract at 40 μg/mL.

Soil

Soil was obtained from Sandoz Crop Protection (Gilroy, CA); its reported characteristics are pH, 7.5; cation exchange capacity, 14.6 mequiv/100 g; organic carbon content, 0.1%; water content, 2.6%; sand, 57.6%, silt, 21.8%; and clay, 20.6%.

Solvents

Solvents were distilled-in-glass and pesticide grade and were obtained from Baxter Scientific (McGaw Park, IL).

Procedure for MAE

MAEs were performed with a MES-1000 microwave sample extraction system (CEM Corporation, Matthews, NC), described in reference 1.

Extraction was as follows: a 5 g portion of soil was accurately weighed in an aluminum dish and transferred quantitatively to the Teflon-lined extraction vessel. To prepare wet samples, the calculated volume of water was added to the sample in the extraction vessel and allowed to equilibrate with the matrix for ca 10 min. A solution containing the test compounds and the 6 surrogate compounds was added to each sample immediately before solvent (30 mL) was added. After ensuring that a new rupture membrane was in place, the extraction vessel was closed. Extractions were performed at 50° or 145°C for 5 or 20 min at 100% power. After extraction, vessels were allowed to cool to room temperature for ca 20 min before they were opened. The supernatant was filtered through glass wool prewashed with solvent and then combined with the 2 to 3 mL solvent rinse of the residue. The extract was concentrated to 1 mL by nitrogen blowdown for analysis by gas chromatography/mass spectrometry (GC/MS). Four solvents, namely 1 + 1 hexane–acetone (HA), 1 + 1 methylene chloride-acetone (MA), 10 + 1 toluene–methanol (MT), and methyl *tert*-butyl ether (MB), were used.

Analysis of Extract

Analyses were performed with a Hewlett-Packard 5890 Series II gas chromatograph interfaced with a Hewlett-Packard 5971A mass spectrometer MSD/DOS Chemstation (Hewlett Packard, Palo Alto, CA) and equipped with a Hewlett-Packard 5973A autoinjector. Samples were introduced via a 30 m length × 0.25 mm id × 0.25 μm film thickness DB-5 fused-silica open-tubular column (J & W Scientific, Folsom, CA) with helium as carrier gas at a flow rate of ca 1 mL/min. Column temperature was held at 40°C for 4 min and then increased at 8°C/min to a final temperature of 300°C, where it was held for 10 min. Injection volume was 1 μL, and injector temperature was 250°C. Injector was set in splitless mode for 1 min after injection. Electron energy was set at 70 eV, and electron multiplier voltage at 2160 V. Data were acquired at 1 s/scan (scanning range was 35–500 amu). The instrument was tuned daily with decafluorotriphenyl phosphine (DFTPP) introduced via the gas chromatograph inlet. A 5-point internal standard calibration using standards at 5, 10, 25, 50, and 100 μg/mL was performed daily to establish the GC/MS linear range. Six internal standards were spiked into every calibration standard and sample extract that was analyzed by GC/MS. For quantitation, we used average relative response factors from multilevel calibration.

Safety

The microwave unit, which incorporates several safety features described in reference 1, must be operated in accordance with the manufacturer's recommended operating safety instructions. A new rupture membrane per vessel should be used for each extraction. Should the membrane rupture because of in-

Table 1. Compounds investigated in this study

Group 1: Neutral Compounds

1. Methyl methanesulfonate
2. Ethyl methanesulfonate
3. Bis(2-chloroethyl)ether
4. 1,3-Dichlorobenzene
5. 1,4-Dichlorobenzene
6. 1,2-Dichlorobenzene
7. Benzyl alcohol
8. Bis(2-chloroisopropyl)ether
9. Hexachloroethane
10. N-Nitroso-dipropylamine
11. Nitrobenzene-d_5[a]
12. Acetophenone
13. Nitrobenzene
14. Isophorone
15. Bis(2-chloroethoxy)methane
16. 1,2,4-Trichlorobenzene
17. Hexachlorobutadiene
18. 1,2,4,5-Tetrachlorobenzene
19. Hexachlorocyclopentadiene
20. 2-Fluorobiphenyl[a]
21. Dimethyl phthalate
22. 2,6-Dinitrotoluene
23. Dibenzofuran
24. Pentachlorobenzene
25. 2,4-Dinitrotoluene
26. 2-Chlorophenylphenyl ether
27. Diethyl phthalate
28. 4-Bromophenylphenyl ether
29. Hexachlorobenzene
30. Pentachloronitrobenzene
31. Di-n-butyl phthalate
32. Butyl benzyl phthalate
33. Bis(2-ethylhexyl)phthalate
34. Di-n-octyl phthalate

Group 2: PAHs

1. Naphthalene
2. 2-Methylnaphthalene
3. 2-Chloronaphthalene
4. 1-Chloronaphthalene
5. Acenaphthylene
6. Acenaphthene
7. Fluorene
8. Phenanthrene
9. Anthracene
10. Fluoranthene
11. Pyrene
12. 4-Terphenyl-d_{14}[a]
13. Benzo(a)anthracene
14. Chrysene

15. 7,12-Dimethylbenz(a)anthracene
16, 17. Benzo(b + k)fluoranthene
18. Benzo(a)pyrene
19. 3-Methylcholanthrene
20. Indeno(1,2,3-cd)pyrene
21. Dibenzo(a,h)anthracene
22. Benzo(g,h,i)perylene

Group 3: Basic Compounds

1. 2-Picoline
2. Aniline
3. N-Nitrosopiperidine
4. α,α-Dimethylphenethylamine
5. 4-Chloroaniline
6. N-Nitrosodibutylamine
7. 2-Nitroaniline
8. 3-Nitroaniline
9. 1-Naphthylamine
10. 2-Naphthylamine
11. 4-Nitroaniline
12. 1,2-Diphenylhydrazine
13. Phenacetin
14. 4-Aminobiphenyl
15. Pronamide
16. Benzidine
17. p-Dimethylaminoazobenzene
18. 3,3'-Dichlorobenzidine
19. Dibenzo(a,j)acridine

Group 4: Phenolic Compounds

1. 2-Fluorophenol[a]
2. Phenol-d_5[a]
3. 2-Chlorophenol
4. Phenol
5. 2-Methylphenol
6. 4-Methylphenol
7. 2-Nitrophenol
8. 2,4-Dimethylphenol
9. 2,4-Dichlorophenol
10. 2,6-Dichlorophenol
11. 4-Chloro-3-methylphenol
12. 2,4,6-Trichlorophenol
13. 2,4,5-Trichlorophenol
14. 2,4-Dinitrophenol
15. 4-Nitrophenol
16. 2,3,4,6-Tetrachlorophenol
17. 2,4,6-Tribromophenol[a]
18. 4,6-Dinitro-2-methylphenol
19. Pentachlorophenol

Group 5: Acid

1. Benzoic acid

[a] Surrogate compound.

creased pressure inside individual vessels, the solvent vapor is unlikely to leak into the cavity, because all vessels are connected to a containment vessel via the solvent rupture vent tube. To prevent pressure buildup inside individual vessels, wet samples should not be extracted simultaneously with dry samples; when extracting 12 samples simultaneously, they should be either all dry or all wet. Likewise, solvent blanks should not be heated together with samples that are to be extracted by MAE, because the former will heat faster than the latter.

Statistical Analysis

Statistical analyses were performed by using the Statgraphics Plus Version 5 (STSC, Inc., Rockville, MD) commercial software package. For each group of compounds, analysis of variance and multiple comparisons of means were performed at the 95% confidence level.

Results and Discussion

At present, 118 semivolatile compounds are listed in EPA Method 8250 (Revision 1, November 1992). Of these, we initially selected 92 compounds for our experimental work; the other 26 compounds were OCPs and PCBs, which we investigated separately with the compounds listed in Method 8081. From the 92 semivolatile compounds, we are reporting data for only 89 compounds. Benzo(*b*)fluoranthene and benzo(*k*)fluoranthene could not be resolved on the DB-5 column, and we are therefore reporting only one set of numbers for both compounds. We deleted *N*-nitroso-dimethylamine, which was difficult to separate from the solvent under the GC conditions used, and *N*-nitroso-diphenylamine, which decomposed in the gas chromatograph inlet to diphenylamine; thus, the latter 2 compounds could not be reliably quantitated by Method 8250 without separate experiments being conducted for each compound. We also investigated 6 surrogate compounds recommended by EPA for use with Method 8250 compounds; thus, the total number of compounds for which we are reporting data is 95.

To facilitate data interpretation, the 95 compounds were divided into 5 groups as follows (Table 1): neutrals (34 compounds), PAHs (22 compounds), basic compounds (19 compounds), phenolic compounds (19 compounds), and acid (1 compound). Recovery data for each compound are included in supplementary material available from the authors.

Neutral Compounds

Figure 1 shows recovery means and 95% confidence intervals for the means for the 34 compounds as a function of matrix [solvent (HA, MA, MT, MB), solvent/dry soil suspension (DS), and solvent/wet soil sus-

pension (WS)], separately for each solvent. Average recoveries (across all 34 compounds) were significantly higher from solvent-alone extracts, followed by the solvent/dry soil and then the solvent/wet soil suspension extracts for all but the MT solvent combination. For MT solvent combination, average recoveries for solvent-alone extracts and solvent/dry soil suspension extracts were not significantly different; however, MT was the only solvent combination of the 4 tested for which average recoveries from the solvent/wet soil suspension extracts were significantly lower than those from the solvent/dry soil suspension extracts.

Figure 2 shows recovery data as a function of time (5 and 20 min), temperature (50° and 145°C) and compound (1 through 34). Raising the temperature from 50° to 145°C resulted in significantly lower overall recoveries, with extraction at 145°C producing lower recoveries. Nonetheless, when recoveries were plotted as functions of compounds, all but 3 compounds (methyl methanesulfonate, ethyl methanesulfonate, and hexachlorocyclopentadiene) had mean recoveries >80%. Mean recoveries of the 3 compounds were 77–79%.

Among 4 solvent combinations, HA appeared to work best for neutral compounds regardless of other factors (soil, time, and temperature).

PAHs

Figures 3 and 4 summarize recovery data for the 22 PAHs. No overall matrix effect could be found (Figure 3), except for MA, for which adding soil (dry or wet) to the solvent significantly reduced average recoveries. However, for this solvent combination, there was no significant difference between recoveries from the solvent/dry soil and the solvent/wet soil suspension extracts. With MB, recoveries were significantly lower from the solvent/wet soil suspensions than from the solvent/dry soil suspensions.

As shown in Figure 4, neither time nor temperature had an overall effect on recoveries. The 4 solvents performed equally well and gave mean recoveries >80%, probably because of the generally high stability of PAHs.

Basic Compounds

Figures 5 and 6 summarize recovery data for the 19 basic compounds. Compared with recoveries of neutral compounds and PAHs, mean recoveries of basic compounds had a wider range, from 95% for MA (solvent only) to 55% for MB/dry soil suspension. Time had no significant effect on recoveries, but temperature did. Average recoveries from samples exposed to 145°C were significantly lower than those from samples exposed to 50°C. Two compounds, α,α-dimethyl-phenethylamine and benzidine, were most affected by matrix, time, and temperature. For example, benzidine
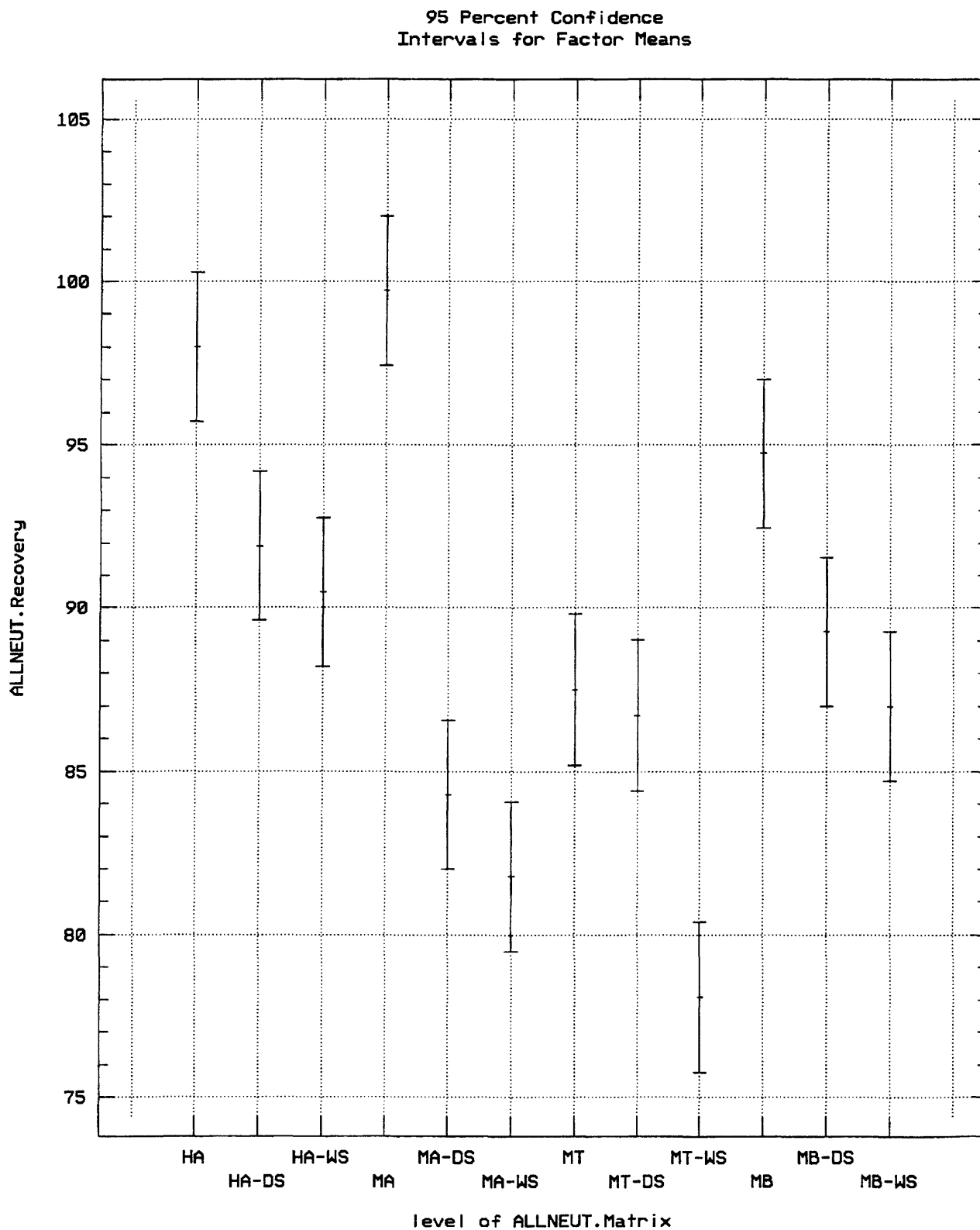
Figure 1.   Recovery as a function of matrix for 34 neutral compounds: means and 95% confidence intervals for factor means.
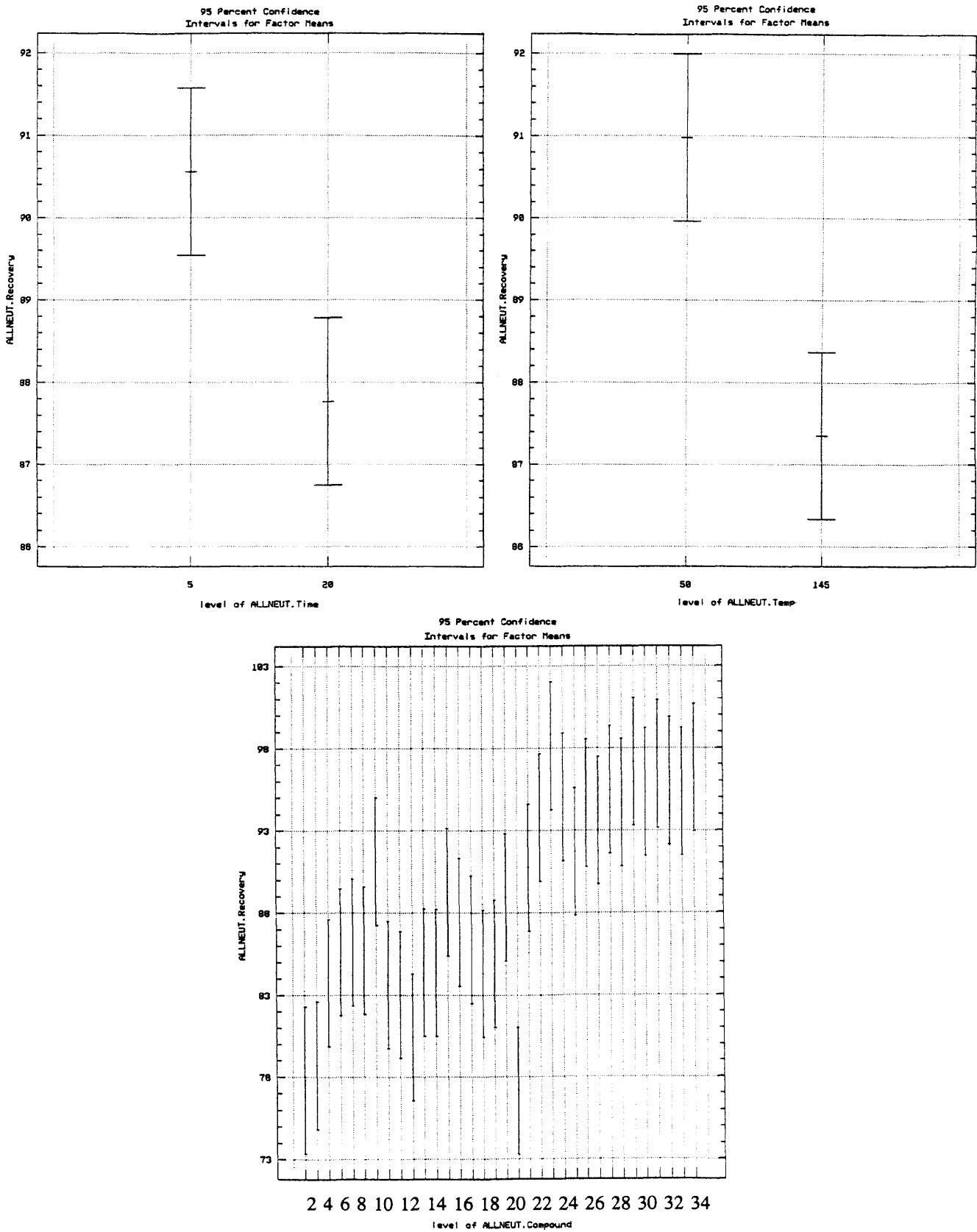
Figure 2.   Recovery as a function of time, temperature, and compound for 34 neutral compounds: means and 95% confidence intervals for factor means. Compounds are arranged in order of elution from the GC column. For compound number, refer to Table 1.
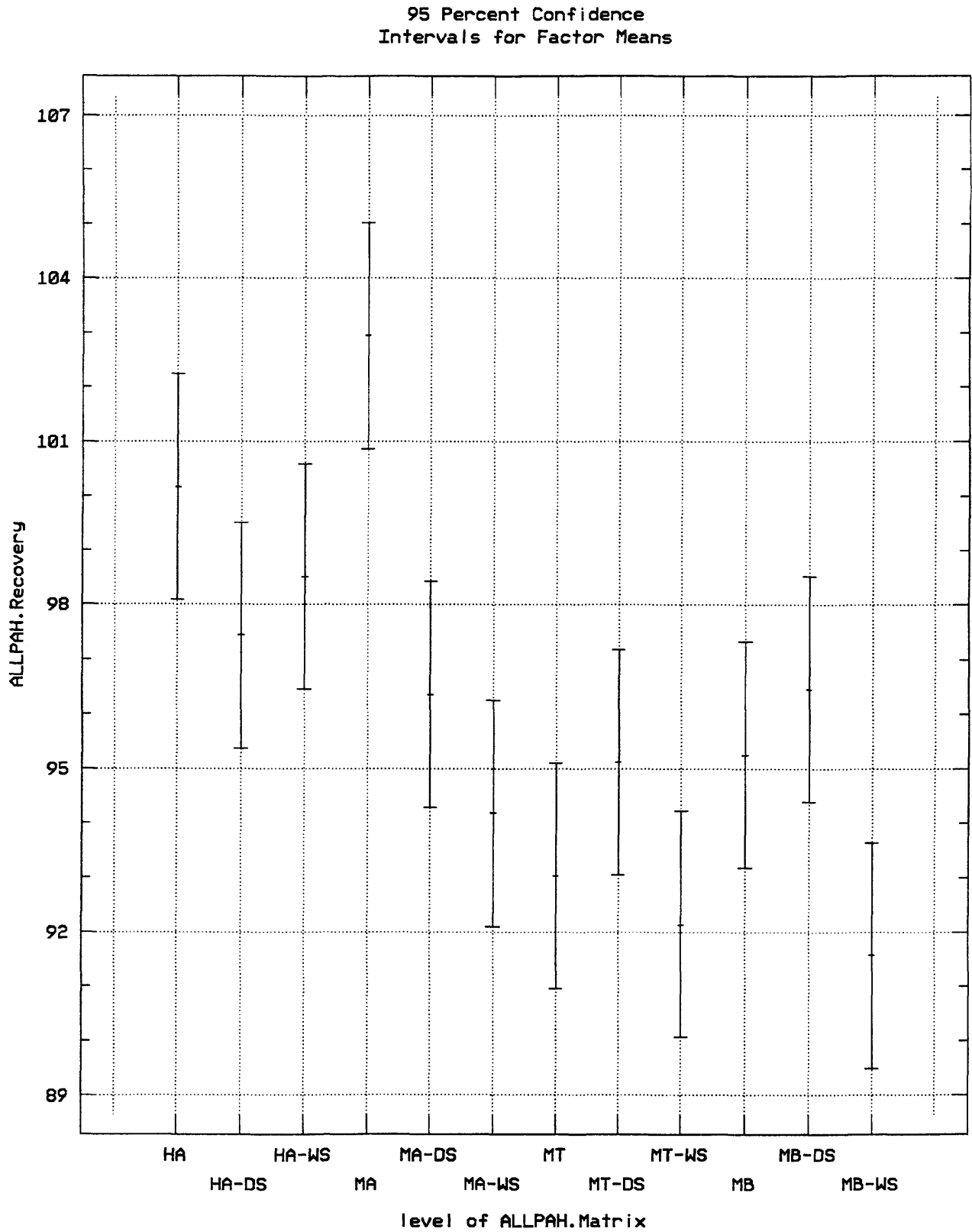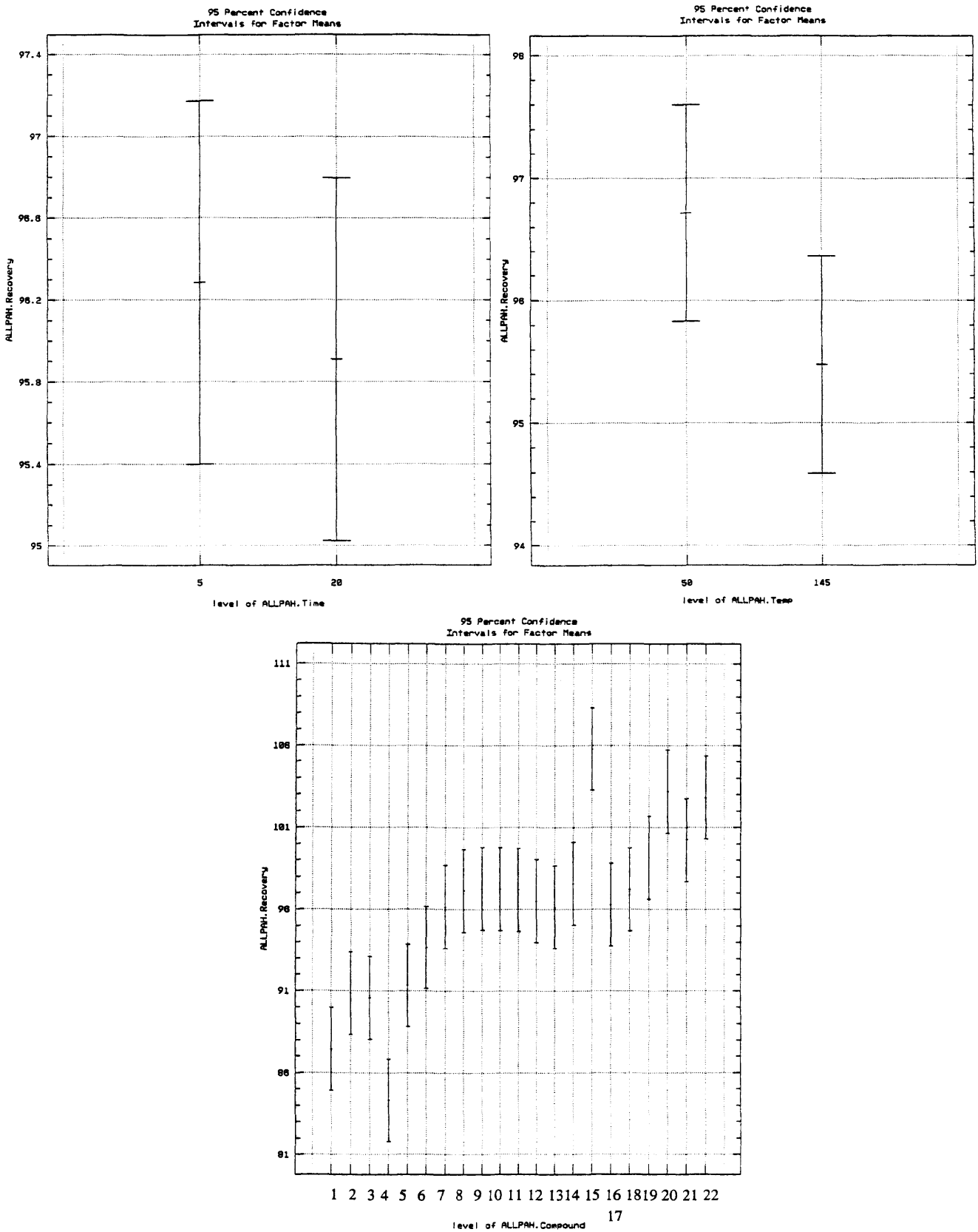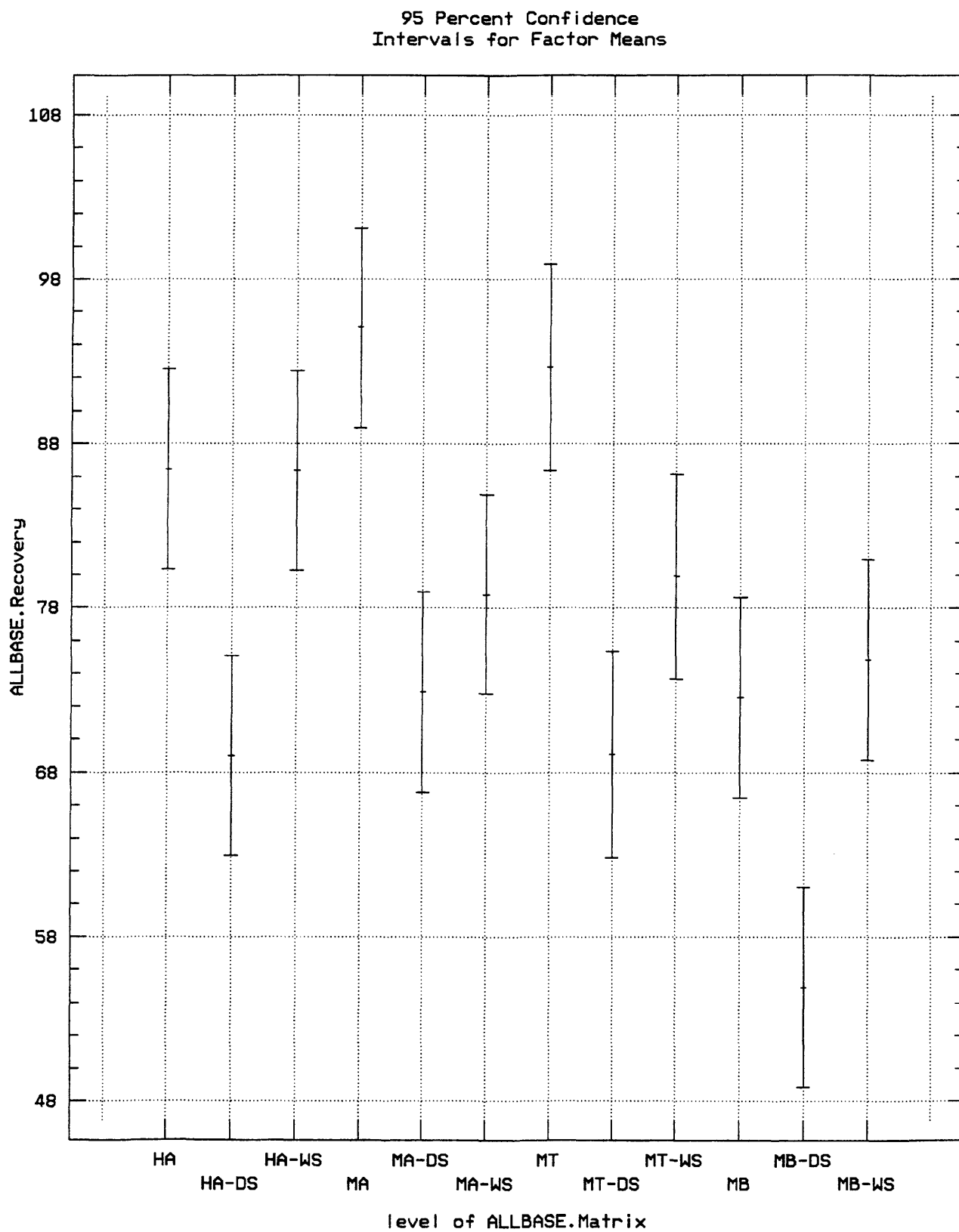
95 Percent Confidence
Intervals for Factor Means

Figure 3. Recovery as a function of matrix for 22 PAHs: means and 95% confidence intervals for factor means.

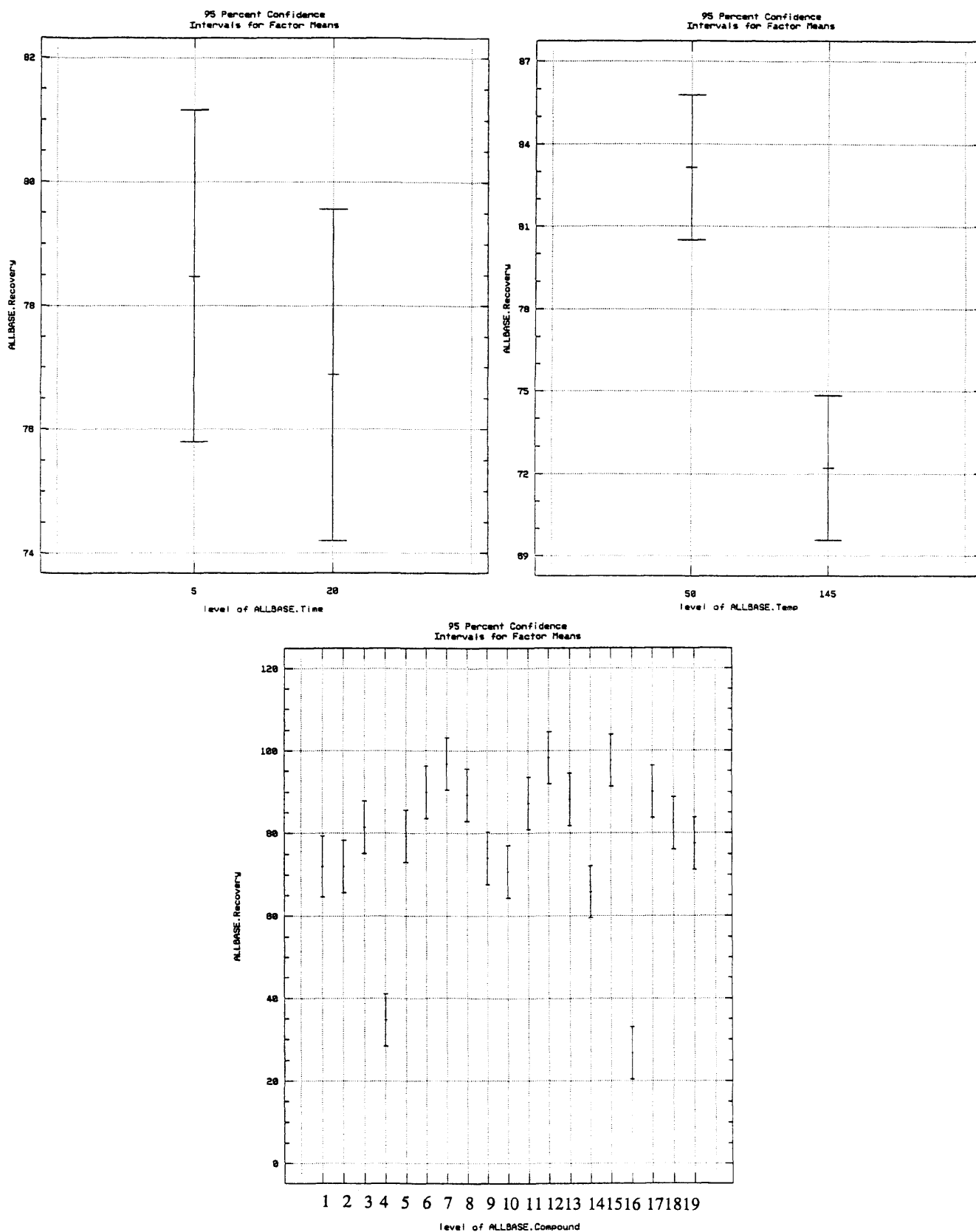**Figure 4.   Recovery as a function of time, temperature, and compound for 22 PAHs: means and 95% confidence intervals for factor means. Compounds are arranged in order of elution from the GC column. For compound number, refer to Table 1.**
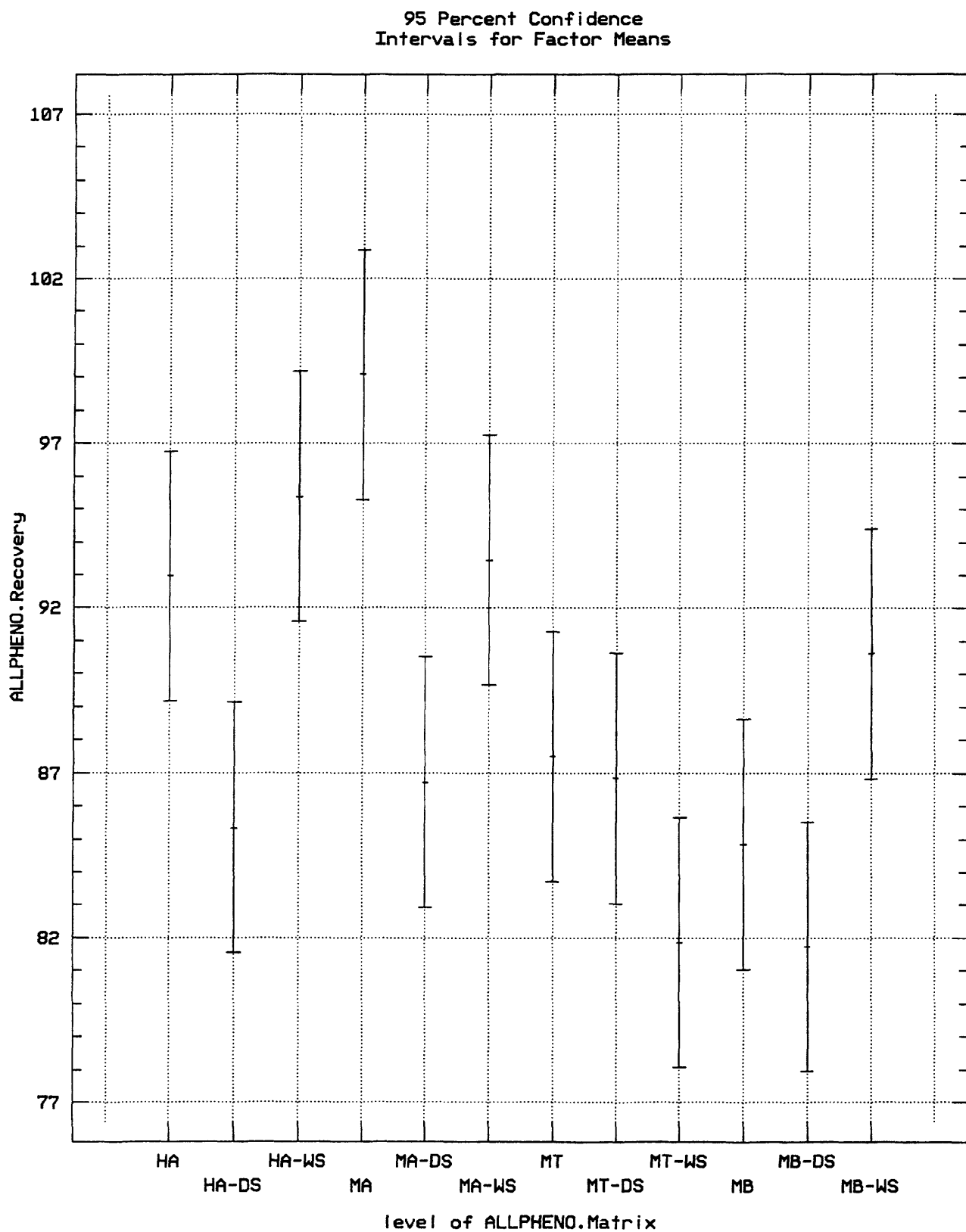
95 Percent Confidence
Intervals for Factor Means

Figure 5. Recovery as a function of matrix for 19 basic compounds: means and 95% confidence intervals for factor means.

Figure 6.   Recovery as a function of time, temperature, and compound for 19 basic compounds: means and 95% confidence intervals for factor means. Compounds are arranged in order of elution from the GC column. For compound number, refer to Table 1.

95 Percent Confidence
Intervals for Factor Means

Figure 7. Recovery as a function of matrix for 19 phenolic compounds: means and 95% confidence intervals for factor means.
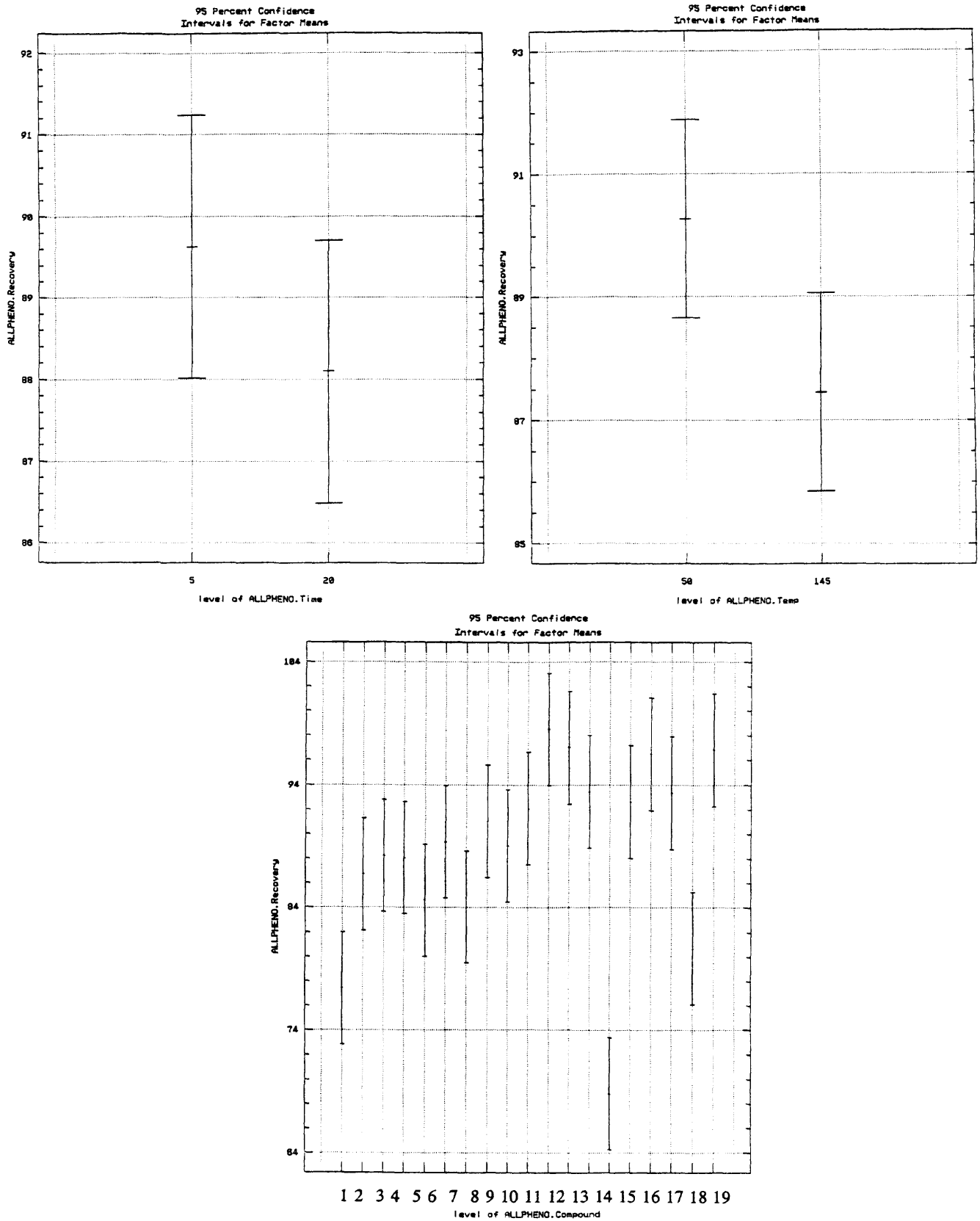
Figure 8.   Recovery as a function of time, temperature, and compound for the 19 phenolic compounds: means and 95% confidence intervals for factor means. Compounds are arranged in order of elution from the GC column. For compound number, refer to Table 1.
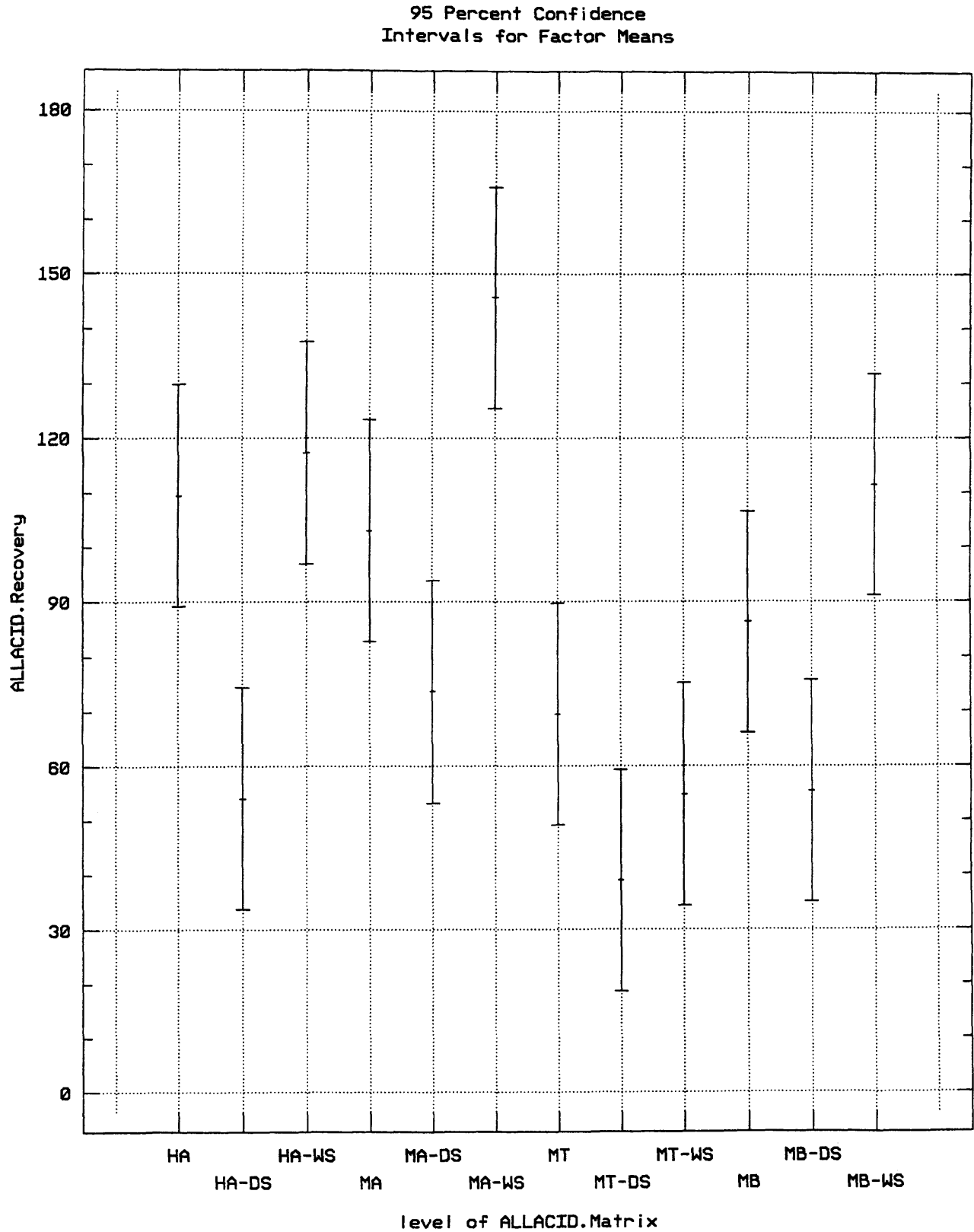
Figure 9. Recovery as a function of matrix for benzoic acid: means and 95% confidence intervals for factor means.

recovery was 88% when heated at 50°C, and only 30% when heated at 145°C in MA alone. When dry or wet soil was present, benzidine recoveries (for the 5 min time) dropped from 21% at 50°C to 1% at 145°C. This behavior of benzidine is not surprising; catalytic reactions in the presence of soil may have contributed to these low recoveries.

Of the 19 basic compounds, 11 had mean recoveries >80%. Recoveries were higher from the solvent/wet soil suspensions than from the solvent/dry soil suspensions.

Phenolic Compounds

Figures 7 and 8 summarize recovery data for 19 phenolic compounds. Mean recoveries were >80% for all solvents and solvent/soil suspension combinations, and neither time nor temperature seemed to have a significant effect on recovery. Except for MT, other solvent combinations seemed to give recoveries that were 6–10% higher when water was present in the soil matrix than when dry soil suspensions were used. 2-Fluorophenol, 2,4-dinitrophenol, and 4,6-dinitro-2-methylphenol gave lower recoveries. We have reported

previously (2) that dinitrophenols gave lower recoveries when MAE was used, possibly because of catalytic reactions with soil components.

Benzoil Acid

The recovery data for benzoic acid are presented in Figures 9 and 10. There is significantly more spread in the recovery data for benzoic acid (Figure 9) than for other compounds, although neither temperature nor heating time appeared to have significant effect on recovery (Figure 10). The wide confidence intervals are a function of the small sample size in each case, because this group includes only one compound.

Conclusions

Overall, the solvent combination HA (1 + 1) seems to be the best for the compounds and matrixes investigated, with recoveries >80%, except for basic compounds and benzoic acid in the solvent/dry soil suspension experiments. Increasing the extraction time from 5 to 20 min did not increase recoveries; in fact, recoveries of neutral compounds decreased slightly at the longer extraction time. Increasing the temperature from
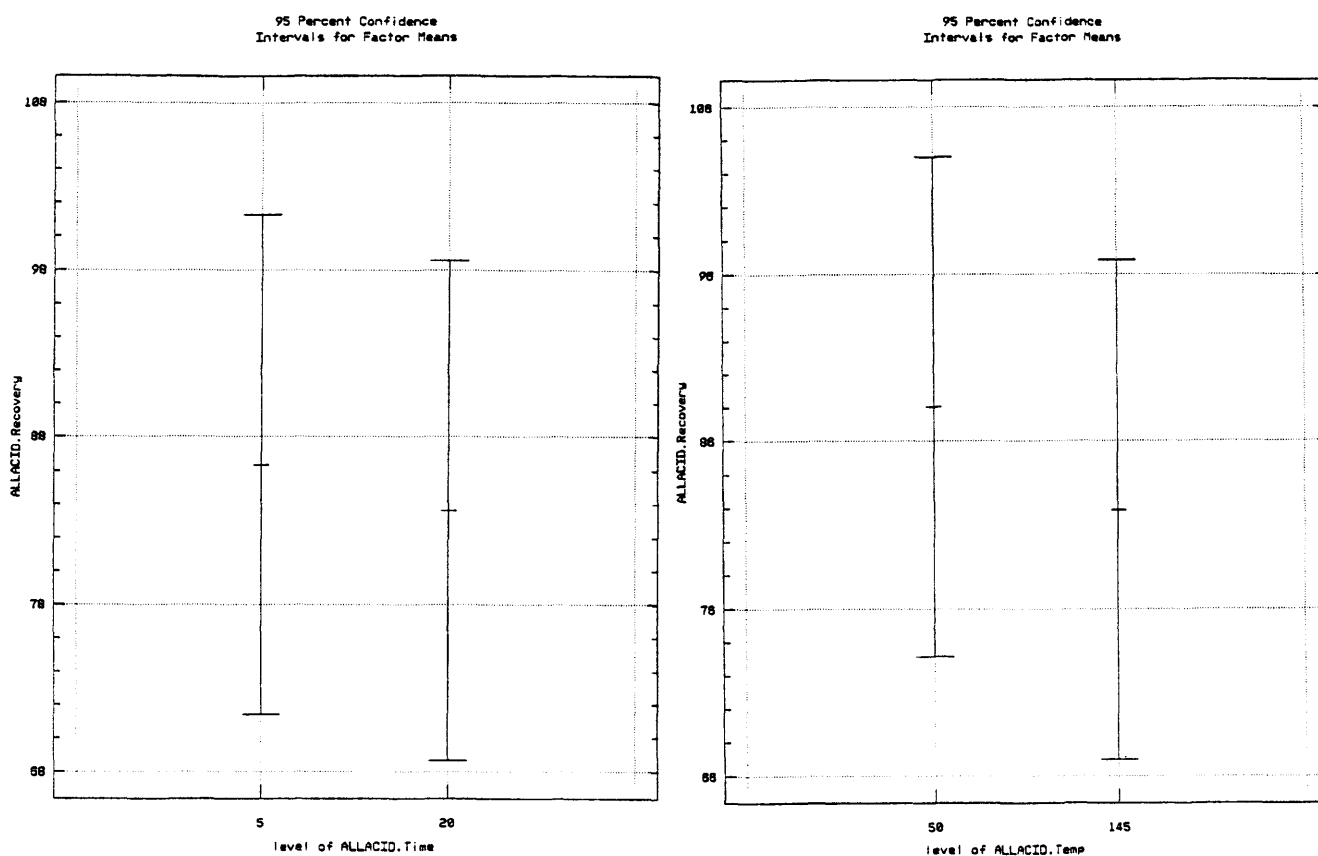
Figure 10.   Recovery as a function of time and temperature for benzoic acid: means and 95% confidence intervals for factor means.

50° to 145°C decreased recoveries of basic compounds by about 10%. Recoveries of basic compounds, of benzoic acid (and probably other organic acids as well), and at least to some extent of phenolic compounds from the solvent/wet soil suspensions are higher than those for solvent/dry soil suspensions. The reason may be that, in solvent/wet soil suspensions, polar sites in the (formerly dry) soil are already occupied by water molecules before other polar molecules have a chance to compete for the site. However, the data are from freshly spiked materials. At present, it is not known to what extent these results could be duplicated with real-world samples.

Notice

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Acknowledgments

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