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Efficiencies of Liquid–Liquid Extraction, Carbon, and XAD-2 Absorption in Isolating Organic Compounds from Environmental Sources

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The Illinois Environmental Protection Agency, long aware of the problems associated with organic pollutants, has developed a program to characterize the organic compounds in industrial discharges (SOMANI et al. 1978, 1979a) and to assess the mutagenic and toxic potential of the discharges (SOMANI et al. 1979b). JANARDAN & SCHAEFFER (1979a) have shown that the quantification of trace organic compounds depends on the efficiency and reproducibility of the sequences used for their collection, recovery and analysis. The present report provides new statistical methods to compare the efficiencies of three sequences to provide reliable data.

MATERIALS AND METHODS

Samples. Samples were collected using an all glass and teflon three-channel positive displacement pump designed for this purpose (TIGWELL & SCHAEFFER 1979). Fifteen liters of effluent, continuously drawn into the pump at a rate of 0.045 L/min through a single teflon intake line, provided equally partitioned flows to cleaned 5-L glass reservoirs for subsequent liquid - liquid extraction (A), and to columns packed to 15 mL bed volume with activated granular carbon (C) or clean (JUNK et al. 1974) XAD-2 (X) resin.

Sample Preparation. Columns were eluted (VAN ROSSUM & WEBB 1978, SOMANI et al. 1979C) by adding nanograde acetone (30 mL) to the column, eluting until the acetone level reached the top of the bed, and adding chloroform (120 mL). Solvents were removed in a Kuderna-Danish (K-D) apparatus until only water and absorbates remained. Water was removed by K-D distillation of an acetone (40 mL, 3 x 20 mL) azeotrope to 5.0-0.5 mL.

The aqueous sample was extracted after pH adjustment (to pH 11 with 6N NaOH and subsequently to pH 2 with 6N HCl) with nanograde dichloromethane (1 x 400 mL, 2 x 200 mL). Solvent extracts were concentrated as above.

Gas chromatography/mass spectrometry. A Varian 2700 series gas chromatograph equipped with a water jacketed septum cooler and a 2.5 m x 6.4 mm OD x 2 mm ID coiled glass column packed with 1.5 percent SP-2250/1.95 percent SP-2401 on 100/120 mesh

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Supelcoport was temperature programmed from 65-250°C at 4°C/min, with the detector held isothermally at 250°C, and a helium carrier gas flow of 20 mL/min. The g.c. was interfaced with an all-glass jet separator to a Dupont 21-491B double focusing low resolution spectrometer equipped with an electron impact source and Incos data system. Details of the analyses have been given by us previously (TEECE et al. 1979, SOMANI et al. 1979 c).

RESULTS AND DISCUSSION

Using these methods, the compounds given in Table 2 were identified. The efficiencies of these methods can be estimated using capture-recapture statistical methods widely employed in biology and medicine (GOLDBERG & WITTES 1978), but seldom in chemistry.

Compounds are to be identified by k(=3) sequences at each of (S + 1) times (S>0), in such a way that a compound which is really present in the source is eventually identified (no false positives). If $p_{i,j}$ is the probability that the jth sequence detects a compound at the ith time, and p_i is the probability that the sequences pick up the compound at the ith time, then $p_{i,j} = 1-q_{i,j}$ is the sensitivity of the jth sequence at the at ith time, and $p_i = 1-q_i$ is the sensitivity of the sequences at the ith time. Letting $n_{i,j}$ be the number of compounds identified at the ith time by the jth sequence, and n_i be the number of compounds identified in the ith time, then $n_i < \sum^{k} n_{i,j}$. The estimated total number of compounds in the ith source, N_i, is the solution of (Darroch 1958):

$$(1) \prod_{j=1}^{k}(N_i - n_{i,j}) = (N_i + 1)^{k-1} (N_i - n_i)$$

with an estimated standard error of

$$(2) \{N_i((1 - \hat{p}_i)^{-1} + (k-1) - \sum_{j=1}^{k}(1 - \hat{p}_{i,j})^{-1})\}^{1/2}$$

where $\hat{p}_{i,j} = n_{i,j}/\hat{N}_i$, $\hat{p}_i = n_i/\hat{N}_i$, and k is the number of sequences.

For k = 3, N_i is solved for iteratively. (A computer program in BASIC is available from DJS.) Equations for the estimated standard errors on $p_{i,j}$ and p_i when k=3 are given by:

$$(3) \text{SE}(\hat{p}_{i,m}) = \{(p_{i,m}(1-\hat{p}_{i,m})(1-\hat{p}_{i,m+1}))/(\hat{N}_i\hat{p}_{i,m+2})\}^{1/2}$$

for m = 1,2,3 (e.g., A, C, X). If m+1=4, it is taken as 1. If m+2=5, it is taken as 2.

TABLE 1. Observed and Estimated Compound Abundances

	Source						
	1a*	1b	1c	2a	2b	2c	t
Method	Observed Frequencies, n_i						
A	3			11			13
C	21	12	10	11	7	7	29
X	19	6	15	12	4	10	27
AC	0			0			0
AX	1			0			1
CX	8	4	5	3	1	3	8
ACX	0			2			3
Observed Total	34	14	20	28	10	14	56
Total, $\hat{N}$	52	18	29	52	19	21	102
SE $(\hat{N})$	10	4	7	13	9	6	18
Estimated Sensitivities							
$\hat{p}_A = n/\hat{N}_A$	0.06			0.21			0.13
SE $(\hat{p}_A)$	0.04			0.10			0.05
$\hat{p}_C = n_C/\hat{N}$	0.40	0.67	0.34	0.21	0.36	0.33	0.28
SE $(\hat{p}_C)$	0.22	0.19	0.12	0.11	0.24	0.15	0.11
$\hat{p}_X = n_X/\hat{N}$	0.37	0.33	0.52	0.23	0.21	0.47	0.26
SE $(\hat{p}_X)$	0.10	0.14	0.16	0.11	0.15	0.19	0.08
$\hat{p} = N/\hat{N}$	0.65	0.78	0.69	0.54	0.53	0.67	0.55
SE $(\hat{p})$	0.65	0.21	0.19	0.55	0.31	0.23	0.41

* 1a and 2a compare liquid-liquid extraction (A), carbon (C) and XAD-2(X) adsorption, without regard to derivatization status.
1b and 2b compare C and X without sample derivatization.
1c and 2c compare C and X, sample derivatized with Meth-Prep II prior to analysis.
Combines the data from both sources without regard to derivatization status.
Sample 1 is the Illinois River at Peoria, Illinois; sample 2 is a petroleum refinery at Lockport, Illinois.

Observed Total = $n_A + n_C + n_X - (n_{A,C} + n_{A,X} + n_{C,X} + 2\ n_{A,C,X})$

(4) $SE(\hat{p}_i) = \{(\hat{p}_i(1-\hat{p}_i)\sum_{j=1}^{3}\hat{p}_{i,j})/(\hat{N}_i\hat{p}_{i,1}\ \hat{p}_{i,2}\ \hat{p}_{i,3})\}^{1/2}$

The computations are illustrated for the data in column 1b of Table 1 for which k = 2.

$n_C = 12,\ n_X = 6,\ n_{C,X} = 4,\ N = 14$

(1) $\hat{N} = (12-1)(6-1)/(4-1) - 1 = 18$

Hence, $p_C = 12/18 = 0.67$, $p_X = 6/18 = 0.33$, $p = 14/18 = 0.78$

(2) $SE(\hat{N}) = \{18((1-0.78)^{-1} + (2-1) - (1-0.33)^{-1} - (1-0.67)^{-1})\}^{1/2} = 4$

(3) $SE(\hat{p}_C) = \{0.67(1-0.67)/18(0.33)\}^{1/2} = 0.19$

$SE(\hat{p}_X) = \{0.33(1-0.33)/18(0.67)\}^{1/2} = 0.14$

(4) $SE(\hat{p}) = \{(0.78(1-0.78)(0.67+0.33))/18(0.67)(0.33)\}^{1/2} = 0.21$

The last column of Table 1 shows that the efficiencies of the liquid-liquid extraction, and carbon and XAD-2 absorption, methods (without regard to derivatization status) are 13% ± 5%, 28% ± 11% and 26% ± 8%, respectively. This agrees with our recent estimate (JANARDAN & SCHAEFFER 1979a) that the data heretofore reported (SHACKELFORD & KEITH 1976, SOMANI et al. 1978, 1979a,c, TEECE et al. 1979) represent 5 to 20% of the compounds actually present in the sample.

These findings do not agree with CHRISWELL et al.'s (1977) observations on the recovery of model compounds from tap water where it was found that ". . . resin is superior to the activated carbon for isolating organic compounds from water". For these data, $N=84$, $\hat{N}=86\pm2$, $\hat{p}_C=0.81$, $\hat{p}_X=0.91\pm0.03$, and

$\hat{p}=0.98\pm0.02$, indicating a slight advantage to using XAD-2.

While factors such as differences between laboratories, compounds or data analysis (statistical) techniques probably contribute to the observed differences, matrix effects are also significantly involved. Thus, the standard errors in Table 1t, which are one-third of the average efficiency, indicate that all the sequences studied by us are highly variable. Consequently, no single sequence is adequate for characterizing a complex sample of unknown composition. The low recovery and high variability preclude accurately relating the analytical values determined for the (general) individual compound to the levels actually present at the source for the reasons given by us previously (JANARDAN & SCHAEFFER 1979a). Thus, the analytical values determined for these compounds provide order of magnitude estimates of the environmental levels of the identified compounds, not precise values. (This may be less true of methods designed to identify specific (classes of) compounds where matrix effects have been specifically considered.)

Comparing the values of $\hat{N}$ in columns 1a and 2a with those (untabled) obtained after deleting method A (48±9 and 25±5), respectively, shows that the total recovery by C plus X is about the same as that of A, C, X. For sample 1, method A contributes very little to the predicted total number of compounds (52-48=4), while for sample 2 the contribution is substantial (52-25=27). Even here, however, method A is very inefficient (21%±10%), suggesting that the numeric constribution may be artifactual. Consequently, Method A should be replaced by a more efficient method, such as an additional sorbent or different extraction procedure.

Comparing columns 1b with 1c and 2b with 2c, we see that derivatization with Meth Prep II prior to analysis increases the estimated number of compounds, $\hat{N}$, present in the samples. Comparison of the estimated number of compounds found in the underivatized (1b and 2b) and derivatized (1c and 2c) samples with each other or with the estimates obtained above without regard to derivatization status, confirms that the efficiencies of the sequences are improved by derivatization. This suggests that, at least in our hands, a general improvement in recovery will be effected by derivatization with Meth Prep II.

Taken together, these results provide quality control information which shows that current procedures are inadequate for broad-based monitoring. Changes in sample collection, extraction and work-up, derivatization, and analysis are clearly needed. These results clearly show that such changes will be difficultly discovered by trial and error, and systematic procedures such as LAUB & PURNELL's (1976) optimization of gas chromatography or Simplex Optimization (MORGAN & DEMING 1974) need to be envoked. Once the optimization experiments define a better procedure, capture-recapture analysis can be applied to measure the effectiveness of the changes. These statistical methods provide valuable information to the analyst who might, for example, find that using an additional sequence is more practical than engaging in the extensive procedural studies and modifications needed to optimize a single sequence. The loss of chemical information uncovered here may be important as studies on the toxicological significance of these complex wastes continues (SOMANI et al. 1979b).

TABLE 2. Compounds Identified

Compounds	1A*	1C	1CD	1X	1XD	2A*	2C	2CD	2X	2XD
o-bromoanisole						X				
butyl acetate		X								
butyl (carbobutoxymethyl) phthalate		X		X			X			
diacetone alcohol		X		X			X	X	X	X
1,7-dimethoxynaphthalene										X
dimethoxyphenylethane							X			
dimethyl adipate			X		X					
dimethylphthalate			X							
2,5-dimethylpiperazine								X		
dioctyladipate				X	X					
dioctylphthlate		X	X	X	X	2-5	X	X	X	X
2,6-di-t-butyl-p-benzoquinone		X								
di-t-butylethylphenol		X								
2-ethylheptanoic acid						10				
2-ethyl-1-hexanol					X					
5-ethyl-2-nonanol			X		X					
hydroxypropyl methacrylate										X
2-methoxyethyl acetate										X
1-methyl-2-bromobenzene						2				
methyl butyl phthalate			X		X					
methyl dehydroabietate	2									
methyl dodecenoate					X					
3-(α-methoxy)methylenebenzofuran			X							
methyl fumarate					X					
3-methyl-4-heptanol		X								
methyl isobutyl carbinol					X					
methyl 10-methyl heptadecanoate					X					
methyl 16-methyl heptadecanoate						2				
methyl 4-methyl pentadecanoate			X							
methyl 11-octadecenoate			X							
methyl oleate	4							X		X
methyl palmitate	3				X	5		X		X
methyl pentadecanoate			X		X					
methyl phenyl ketone							X			
methyl phthalate					X					
methyl stearate								X		X
methyl tetradecanoate					X					X
p-nonylphenol		X		X						
5-nor-butyldocosane									X	
nor-heptadecane									X	
nor-hexacosane		X								
nor-pentacosane		X					X			
nor-tetracosane			X							
nor-tricosane		X								
1-octadecanol					X					
octamethylcyclotetrasiloxane								X		
pristane										X
1-tetradecanol						2				
2,6,10,14-tetramethyl-pentadecane						2				
thiophenol							X			
2,3,5-trimethyl-1,4-benzoquionone						1				
toluene						9				
p-toluenesulphonamide						2				
1,2,4-tri-t-butylbenzene		X								
trioctyl phosphate				X						

*Concentrations in ppb.

REFERENCES

CHRISWELL, C.D., R.L. ERICSON, G.A. JUNK, K.W. LEE, J.S. FRITZ, and H.J. SVEC: J. Amer. Water Works Assoc. 69, 669 (1977).

DARROCH, J.N.: Biom. 45, 343 (1958).

GOLDBERG, J.D., and J.T. WITTES: Biom. 34, 77 (1978).

JANARDAN, K.G., and D.J. SCHAEFFER: Anal. Chem. 51, 1024 (1979a).

JANARDAN, K.G., and D.J. SCHAEFFER: To appear in the Proceedings of the Tenth Annual Pittsburgh Conference on Modeling and Similation, April 25-27 (1979b). Pittsburgh: Instrument Society of American in press (1979).

JUNK, G.A., J.J. RICHARD, M.D. GRIESER, D. WITIAK, J.L. WITIAK, M.D. ARGUELLO, R. VICK, H.J. SVEC, J.S. FRITZ, and G.U. CALDER: J. Chrom. 99, 745 (1974).

LAUB, R.J., and J.H. PURNELL: Anal. Chem. 48, 1720 (1976).

MORGAN, S.L., and S.N. DEMING: Anal Chem. 46, 1170 (1974).

SHACKELFORD, W.M., and L.H. KEITH: Frequency of Organic Compounds Identified in Water. EPA 600/4-76-062. Athens, G.A. US Environmental Protection Agency (1976).

SOMANI, S.M., J. JOHNSTON, and D.J. SCHAEFFER: Seventh International Congress of Pharmacology, Paris, France, July 16-21 (1978).

SOMANI, S.M., R.G. TEECE, and D.J. SCHAEFFER: Pharm. Appl. Toxicol. 48, A24 (1979a).

SOMANI, S.M., J. MACK, and D.J. SCHAEFFER: Pharm. Appl. Toxicol. 48, A132 (1979b).

SOMANI, S.M., R.G. TEECE, and D.J. SCHAEFFER: J. Toxicol. Environ. Health. Accepted for Publication (1979c).

TEECE, R.G., S.M. SOMANI, and D.J. SCHAEFFER: American Society for Mass Spectrometry. Twenty-Seventh Annual Conference on Mass Spectrometry and Allied Topics. Seattle, Washington June 3-8 (1979).

TIGWELL, D., and D.J. SCHAEFFER: In Preparation for Anal. Chem. (1979).

VAN ROSSUM, P., and R.G. WEBB: J. Chromatog. 150, 381 (1978).