Letters to the Editor

Screening of Benzodiazepines and Metabolites

Sir:

It is with disbelief that I read the article on quantitative screening of benzodiazepines and metabolites by electron-capture gas chromatography and high pressure liquid chromatography published in the *Journal of Forensic Sciences* (Vol. 24, No. 1, Jan. 1979, pp. 46-54) by Peat and Kopjak.

While I find no fault with the write-up, I do question the analytical validity of the chromatography from which one must derive the analytical results. If one assumes that this is exemplary of the author's best work, then what does the routine work look like? I question how one can produce quantitative or even qualitative results from this type of chromatography. It is not surprising that no data on precision, recovery, or accuracy are presented. I have a great deal of respect for Bryan Finkle and find it hard to believe that he was in any way associated with this publication. It is apparent that, although the authors submitted the paper, the editors should reexamine the criteria for acceptance.

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Authors' Reply

Sir:

Mr. Thoma's criticism of our paper in the July issue describing the analysis of some benzodiazepine drugs and metabolites by electron-capture gas chromatography (GLC-ECD) and high pressure liquid chromatography (HPLC) is spirited but vague. However, constructive comment is useful and in that context the omission of accuracy, precision, and recovery data and the negative blast at the illustrations of chromatograms require a response.

The chromatograms in the published paper illustrated qualitative analyses from actual case samples. Figure 6*C* described an unusually complex case in that both diazepam and flurazepam had been ingested. No attempt was made to produce artificial "best-work" with aesthetic appeal but deceptive of routine laboratory experience. Figure 1 shows what can be easily achieved under contrived conditions. Chromatogram *A* is from an extract of drug-free whole blood with only the internal standard (flunitrazepam) added, and Chromatogram *B* is from extracted blood to which diazepam and *N*-desmethyldiazepam have been added, each at 500 ng/ml. Chromatogram *C* is the same, except flurazepam and *N*-desalkylflurazepam were the added drugs. The unlabeled peak in each of the chromatograms is an impurity present in drug-free blood obtained from the blood bank. It is noteworthy that both flunitrazepam and prazepam have recently become available for medical use in the United States and should, therefore, be used with discretion as internal standards in case work.

The HPLC procedure is used to assay chlordiazepoxide and its metabolites as described in the published paper; the GLC-ECD method is used for the other common benzodiazepine drugs and metabolites. Tables 1 and 2 provide precision data for the GLC and HPLC methods. Each sample was prepared gravimetrically to contain the target values indicated in the tables. These data were available but not requested for the original publication. The paper did, however, illustrate calibration curves typical of routine, daily work. The

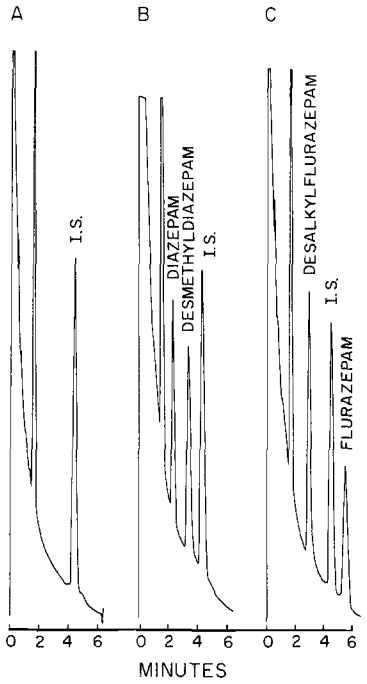

FIG. 1—(A) *Extract of drug-free whole blood with only the internal standard flunitrazepam added* (IS). (B) *Extracted blood to which diazepam and* N*-desmethyldiazepam were added, each at 500 ng/ml.* (C) *The same, except flurazepam and* N*-desalkylflurazepam were the added drugs. The unlabeled peak in each of the chromatograms is an impurity present in drug-free blood obtained from the blood bank.*

TABLE 1—*Precision data for GLC-ECD.*

Drug	*n*	Mean, ng/ml	Standard Deviation	Coefficient of Variation, %
		Within-Run		
Diazepam	10	509	7.2	1.4
N-Desmethyldiazepam	10	510	11.1	2.2
Flurazepam	10	478	8.1	1.7
N-Desalkylflurazepam	10	495	7.5	1.5
		(Target Value: 500)		
		Run-to-Run		
Diazepam	20	496	14.1	2.8
N-Desmethyldiazepam	20	485	12.2	2.5
Flurazepam	20	476	12.6	2.6
N-Desalkylflurazepam	20	488	14.6	3.0
		(Target Value: 500)		

accuracy limits of the procedures can be inferred from the tables, but, more importantly, continuous internal and external proficiency testing has confirmed the acceptability of the methods for qualitative identification and quantitation, for example, through the College of American Pathologists' toxicology program in which the Center for Human Toxicology is both a participant and referee laboratory. The practical limit of sensitivity

TABLE 2—*Statistics for chlordiazepoxide and its metabolites.*

Drug	Concentration When First Analyzed[a]	*n*	Mean	Standard Deviation	Coefficient of Variation, %
		Within-Run			
Chlordiazepoxide	384	10	383	13	3.4
Chlordiazepoxide	220	10	221	15	6.8
Desmethylchlordiazepoxide	271	10	273	12	4.4
Desmethylchlordiazepoxide	133	10	132	9.7	7.4
Demoxepam	303	10	307	19	6.3
Demoxepam	117	10	118	9.4	8.0
		Day-to-Day			
Chlordiazepoxide	805	5	750	54.3	7.2
Chlordiazepoxide	198	5	184	7.3	4.0
Desmethylchlordiazepoxide	692	5	666	51.7	7.8
Desmethylchlordiazepoxide	110	5	103	5.4	5.2
Demoxepam	431	5	424	33.8	8.0
Demoxepam	84	5	78	3.5	4.5

[a] Target value concentration in ng/ml.

for each of the drugs and metabolites is approximately 50 ng/ml when a 1.0-ml sample is analyzed by either method. Absolute extraction-recovery values are irrelevant to the evaluation of any analytical method in which a true internal standard is used, that is, one which parallels the chemistry of the analyte and is added to the original sample as the first step of the analysis. The recovery value only has bearing inasmuch as very poor extraction efficiency may effect sensitivity; however, Mr. Thoma may wish to know that recovery values from plasma and whole blood consistently exceed 85%.

This additional information will amplify the original paper and we trust will satisfy some of Mr. Thoma's questions. We are aware that other toxicologists do use the described procedures, with or without minor modifications, and we would therefore be interested in their opinions concerning the utility of the method.

Thank you for the opportunity to reply.

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