J Forensic Sci, Jul. 1975, Vol. 20, No. 3

J. H. Jerpe,[1] *B.S.; F. E. Bena,*[1] *B.S.; and William Morris,*[2] *B.S.*

GC-Quadrupole Mass Fragmentography of Heroin

The use of internal standards in solutions analyzed by gas chromatography (GC) has become a routine system for simultaneous quantitative and qualitative analysis. Selection of a suitable internal standard has depended upon the following: (1) elution time must not significantly overlap the elution time of drug assayed, and preferably elute later; (2) the detector response must be linear over a wide range of concentrations; (3) a stock solution should be stable for a considerable period of time; and (4) the preparation and synthesis of internal standard should be rapid or the standard should be commercially available.

Recently, a method has been introduced into the field which precludes some of the above-stated requirements for an internal standard and offers the advantage of using the mass spectrometer (MS) sensitivity as a gas chromatograph detector system. The earliest use of this technique was in 1968, when mass fragmentography was introduced and demonstrated the identification of chlorpromazine and its metabolites in human blood [*1*]. Since then, the technique of monitoring a single mass fragment ion as a detector system for the compound of interest has been applied to the quantitation of tetrahydrocannabinol in plasma [*2*]; plasma concentration of lidocaine [*3*]; analysis of steroids, catecholamines, and amino acids [*4*]; quantitation of normorphine in urine [*5*]; quantitation of 5-hydroxyindole-3-acetic acid in cerebrospinal fluid [*6*]; and, recently, a method of measurement of prostaglandin $F_{2\alpha}$ in biological fluids [*7*].

The use of mass fragmentography offers one of the only physical methods for measuring some of these biological chemicals, due to the sensitivity required for detection in the concentrations present in life systems. The method involves the measurement of specific ion intensity ratios between the amount of added internal standard and the compound of interest. Usually one must make a volatile derivative of the compound to facilitate gas chromatographic separation. The use of stable-isotope-labeled molecules is probably the closest approach to an ideal internal standard, because of the nearly identical physical and chemical properties between the molecules. Under most gas chromatographic systems, the compound and its deuterated analogue will co-chromatograph, with the distinction that the molecular ion in the fragmentography pattern will differ in mass by substituted deuterons.

Our intention was to determine the feasibility of the mass fragmentographic determination of heroin in vitro systems, especially as applied to drug laboratory seizures; to monitor the signal output on a programmable multiple ion monitoring

Received for publication 26 Feb. 1974; revised manuscript received 30 Sept. 1974; accepted for publication 17 Nov. 1974.

[1] Forensic Chemists, Mid-Atlantic Regional Laboratory, Drug Enforcement Administration, Washington, D.C.

[2] Applications Chemist, Finnigan Corp., 595 North Pastoria Ave., Sunnyvale, Calif. 94086.

system (PROMIM); to determine the precision of quantitative analysis from data on light-sensitive recording paper; and to estimate the limit of detection of heroin by this method.

Experimental

Since the sources of samples of the drug were crystalline powder from clandestine manufacture, a rigorous separation procedure was not followed (as required for blood extract). (A review of pertinent literature regarding separations of such samples from a difficult matrix usually notes a Sephadex® chromatography column separation of centrifuged light petroleum layers. The deuterated internal standard is added prior to the commencement of separation, as recovery has been estimated to be approximately three parts in four.) The powder, as received, was weighed to the nearest one-hundredth of one milligram on a Cahn electrobalance. Internal standard methanol solution was added by pipet. Approximately one microlitre of the resultant solution was injected into the GC/MS system.

Preparation of Standard Solution

Deuterated heroin, free base, with a melting point of 170.5°C (Argonne National Laboratories) was used for a stock standard solution. The heroin was synthesized directly from morphine with deuterated acetyl chloride. The methanol solutions were injected into a GC/MS system using a 1% OV-1, 4-ft, ¼-in. glass column packed with 80/100 WHP heated to 230°C, coupled to a Finnigan Model 3000 quadrupole mass spectrometer. A PROMIM accessory was used to monitor four specific ion fragments continuously during the GC elution. The intensity of each specific ion fragment was recorded on a separate channel of a multipen recorder. Serially diluted solution concentrations were graphed to determine linearity of response within the magnitude of <1 to >100 ng of sample injected on column.

Quantitation was achieved by comparing the relative peak areas. Also, the precision of the system was investigated by averaging a large number of repeated scans from the recording chart paper. This may be done by excluding PROMIM as a signal monitor and merely using the record mode repeated until the drug has completely eluted.

The scan range may be manually adjusted to permit selection of that portion of the mass range including the molecular ions *m/e* 369 and *m/e* 375 and excluding the remainder of the spectra.

Results and Discussion

Many deuterated internal standards are synthesized by a simple solvent exchange equilibria with deuterated solvent. Repeated extraction with organic solvents results in progressively purer compound. However, it is often necessary to synthesize a deuterated compound by judiciously substituting a deuterated reactant within the series of reactions in such a manner as to introduce deuterons at a particular site. This step is predicated primarily by the fragmentation pathway of the compound in question; benzyllic hydrogens are often chosen as the substitution site. (It has also been shown feasible to use the compound to be measured, modified by introduction of the stable isotope ^{15}N) [*8,9*]. The resultant mass increase of 2 atomic mass units (amu) is suitable for offering a signal of significantly higher mass to which a detector system (variously named acceleration voltage alternation, gas chromatography/mass spectro-

metry accelerating voltage alternation, or programmable multiple ion monitoring) may be tuned. These ions are alternatively focused on the ion detector of the mass spectrometer while the effluent of the gas chromatograph is applied. This alternating focusing must be performed at a rate sufficient for many measurements of the ion signals. This is accomplished by accelerating voltage adjustment in a magnetic sector instrument or by DC/RF adjustment in a quadrupole mass spectrometer.

However, synthesis of heroin resulting in the increase of 6 amu to the molecular ion presents a new problem. We have noted a difference in gas chromatograph retention times between the internal standard and drug. Although the difference is slight (four to six seconds on 3% OV-17 and one to two seconds on 1% OV-1), scans for quantitative purposes must not include the first appearance of a signal. The addition of three protons per acetyl group and increase in mass of 6 parts per 369 evidently changes the partition characteristics of deuterated heroin enough to account for this slight separation. If one uses high performance columns, the separation could preclude quantitation by this method.

The stability of the synthesized internal standard was determined by storing a methanol solution of a mixture of heroin and deuterated heroin at 5°C for six months. The relative peak intensities of this solution showed no change by mass spectrometric scans within this time period. Figure 1 is the computer-stored printout of the mass

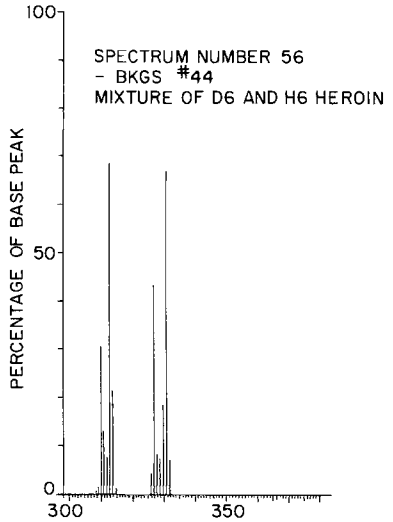

FIG. 1—*Computer printout of spectrum of mixture of deuterated heroin plus heroin.*

spectra and fragmentation pattern for the mixture of heroin and internal standard prepared. Figure 2 is a tracing of the oscilloscope display after adjusting the mass range to cover only the area of ions of interest in the repeat scan mode. The time from the first peak to the end of the scan is approximately one tenth of a second. Therefore, during the total time of elution by suitably adjusting electronics, a series of as many as 300 repeat scans may be made, enhancing the inherent statistics of the determination.

To estimate the precision of data displayed on light-sensitive chart paper, serial

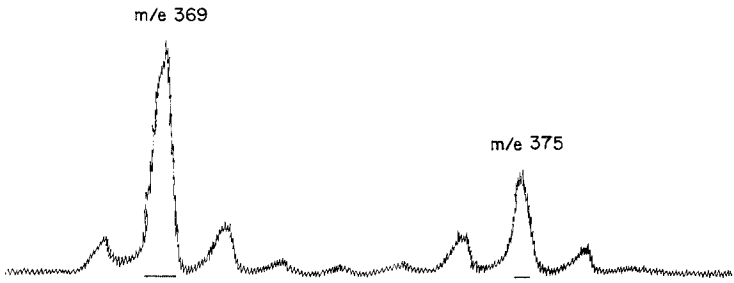

FIG. 2—*Light-sensitive chart paper recording of GC/MS scan of deuterated heroin plus heroin.*

injections were made of a mixture solution approximately four minutes apart. The scans were made in repeat-scan mode, that is, sweep-speed 0.1, record continuous, with the display adjusted to the range of 360 to 380 amu. In this manner, 100 or more scans may be repeated during the total time of elution. The results are shown in Table 1.

TABLE 1—*Precision of replicate sample*[a] *injections.*

No. of *m/e* Ratios Measured	Sample Average
60	2.502
76	2.618
55	2.482
106	2.651
54	2.601
Average	2.571

[a]Same solution, five serial injections.

Figure 3 is a tracing reproduced from an interactive data system (IDS) on which the multiple ion monitor signal response was stored. This system includes a dedicated minicomputer and necessary interfacing electronics. The parent compound molecular ion is *m/e* 369; synthesized internal standard-d_6 molecular ion is *m/e* 375; heroin minus the primary fragment, $CH_2{=}CO$, is *m/e* 327; and internal standard minus the primary fragment is *m/e* 331. The small signal at *m/e* 327 prior to elution of heroin is due to the fragmentation of traces of monoacetyl morphine present. This mass fragmentogram can be plotted in real time (interactive data system, Model 6000) as a computer-reconstructed plot [*10*].

Figure 4 is a plot of responses of the fragments *m/e* 369 and *m/e* 375 versus nanograms injected. The slopes are not parallel, inferring that the response factors for these two ions are different. Apparently the chemical bond of the deuterated radical is not energetically equivalent to acetylated morphine and hence the stability of the ion fragment is not the same. These differences in response are analogous to response factors in various detector systems in gas chromatography.

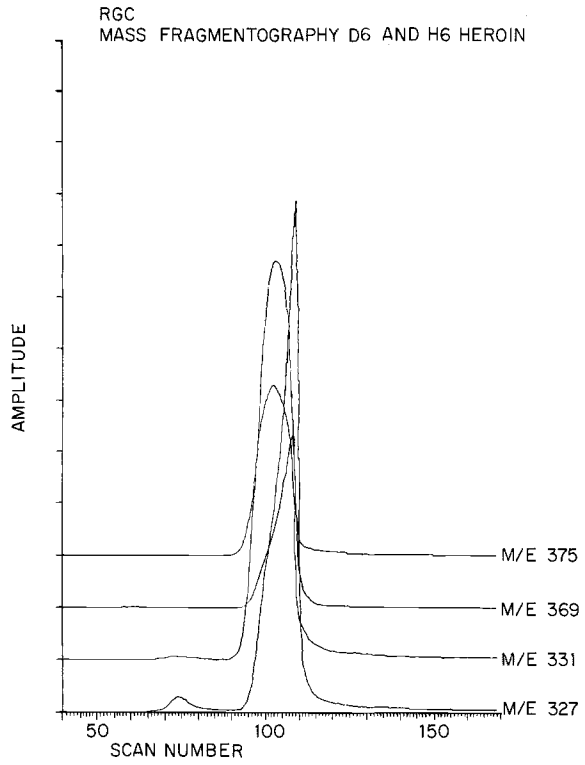

FIG. 3—*Computer printout of PROMIM data of mass fragmentogram.*

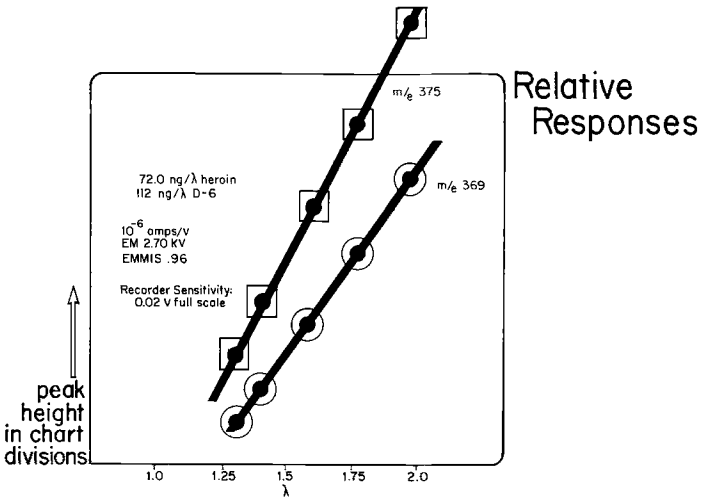

FIG. 4—*Relative responses of molecular ions.*

The system described was used to quantitate heroin samples submitted by a referee investigator in a continuing Association of Official Analytical Chemists (AOAC) study. These samples had been analyzed by laboratories across the United States by cooperating law enforcement agencies. Accuracy was adjudged by weighing approximately 10-mg samples on a Cahn balance, diluting with less than 1 ml of a methanol solution of deuterated standard, and completing assay from the weight/response ratios (Fig. 5). These results are tabulated in Table 2.

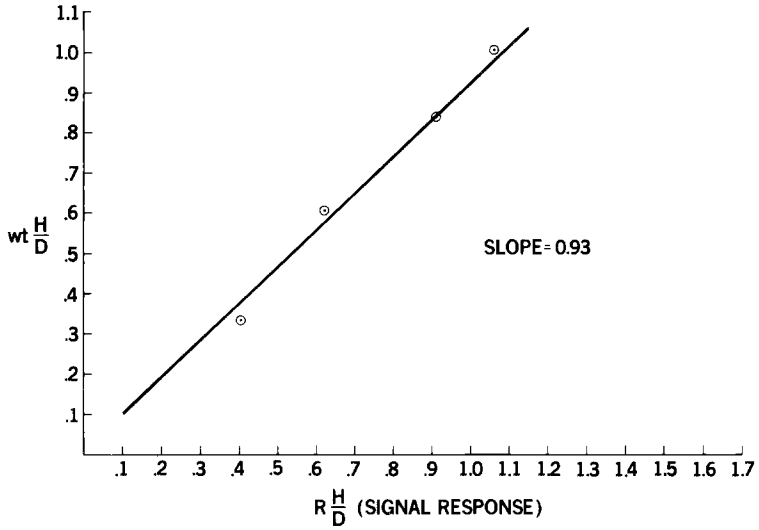

FIG. 5—*Weight ratio of heroin to deuterated heroin* (wt H/D) *versus signal response of heroin to deuterated heroin* (R H/D).

TABLE 2—*Quantitation from recorder data versus gas-liquid chromatography (GLC) assay.*

Chart Paper Ratio, *H/D*	Sample Weight, mg	Sample Weight/ Internal Standard Weight	*H/D*, % purity	AOAC, % purity
1.22	11.96	48	2.5	2.65
5.30	10.98	43	12.0	12.8
3.34	8.25	65	5.1	5.2

H/D = heroin signal intensity/deuterated heroin signal intensity

Summary

Quantitative analysis by quadrupole mass fragmentography is a rapid and accurate method for assay. Its advantage is fundamentally the requirement for very small sample sizes. One must allude to the disadvantage of these techniques in the preparation of a deuterated internal standard (if not commercially available) and operator experience with sophisticated instrumentation.

By extrapolating the signal intensities to the baseline intersection (no response) assuming optimal gas chromatographic conditions, 0.5 ng of heroin injected on column appears to be the limit of detection by the above-referenced equipment.

References

[1] Hammar, C.-G., Holmstedt, B., and Ryhage, R., *Analytical Biochemistry,* Vol. 25, 1968, pp. 532-548.

[2] Agurell, S., Gustafsson, B., Holmstedt, B., Leander, K., Lindgren, J.-E., Nilsson, I., Sandberg, F., and Asberg, M., *Journal of Pharmacy and Pharmacology,* Vol. 25, No. 7, July 1973, pp. 554-558.

[3] Strong, J. M. and Atkinson, A. J., Jr., *Analytical Chemistry,* Vol. 44, No. 14, Dec. 1972, pp. 2287-2290.

[4] Maume, B. F., Bournot, P., Lhuguenot, J. C., Baron, C., Barbier, F., Maume, G., Prost, M., and Padieu, P., *Analytical Chemistry*, Vol. 45, No. 7, June 1973, pp. 1073-1082.

[5] Ebbighausen, W. O. R., Mowat, J., and Vestergaard, P. *Journal of Pharmaceutical Sciences*, Vol. 62, No. 1, Jan. 1973, pp. 146-148.

[6] Bertilsson, L., Atkinson, A. J., Jr., Althus, J. R., Harfast, A., Lindgren, J.-E., and Holmstedt, B., *Analytical Chemistry,* Vol. 44, No. 8, July 1972, pp. 1434-1438.

[7] Kelly, R. W., *Analytical Chemistry,* Vol. 45, No. 12, Oct. 1973, pp. 2079-2082.

[8] Samuelsson, B., Hamberg, M., and Sweeley, C. C., *Analytical Biochemistry,* Vol. 38, Nov.-Dec. 1970, pp. 301-304.

[9] Gaffney, T. E., Hammar, C.-G., Holmstedt, B., and McMahon, R. E., *Analytical Chemistry,* Vol. 43, No. 3, March 1971, pp. 307-310.

[10] Bell, N. W., "Computer Detection of MS Peaks by Real-Time Cross Correlation," presented at the 21st Annual Conference on Mass Spectrometry and Allied Topics, San Francisco, 20 May 1973.

Mid-Atlantic Regional Laboratory
Drug Enforcement Administration
460 New York, Ave., N.W.
Washington, D.C. 20537