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Quantitative Analysis of Illicit Heroin by Selected Ion Monitoring

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ABSTRACT: A method using selected ion monitoring has been developed for the quantitative analysis of illicit heroin. It is accurate and reproducible. Comparing the results obtained by this method and by gas chromatography and high performance liquid chromatography should provide an absolute heroin content within the limits of experimental error.

KEYWORDS: toxicology, heroin, spectroscopic analysis, selected ion monitoring

Gas chromatography (GC) is perhaps the most frequently used technique in determining the quantity of heroin in illicit samples [*1,2*] because it offers minimal sample preparation, high resolution, excellent sensitivity, and accurate and precise results. In countries such as Singapore where legal penalties depend on the quantity of heroin involved and a mandatory death penalty is attracted for trafficking in more than 15 g of pure heroin, GC quantitation alone may not be sufficient when the net heroin content is marginally above 15 g. Theoretically there may be another compound co-chromatographed with heroin, raising doubts about the accuracy of the results and, consequently, about the amount of pure heroin involved. Recently, high performance liquid chromatography (HPLC) has been used for the analysis of heroin but with the same limitations [*3,4*]. Spectroscopic methods usually require tedious extraction procedures and do not necessarily provide the selective identification needed. Selected ion monitoring (SIM), in which a mass spectrometer is used to acquire and record ion current at a certain selected mass per charge value, is the only physical method offering the necessary identification selectivity with quantitative capability [*5*]. This paper seeks to study the feasibility of utilizing SIM in the determination of illicit heroin.

Experimental Procedure

Apparatus

Gas Chromatography/Mass Spectrometry—A Hewlett-Packard 5985B gas chromatography/mass spectrometry system equipped with a 1.8-m (6-ft) by 6.35-mm (¼-in.) outside diameter glass column packed with 3% OV-1 on Chromosorb WHP, 100-120 mesh, was used. The helium flow was 30 mL/min. The injector and column temperatures were 275 and 250°C, respectively. The gas chromatograph was interfaced to the mass spectrometer with a glass jet separator maintained at 275°C. The mass spectrometer (MS) ion source tempera-

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ture was maintained at 200°C and operated at 70 eV. Mass data were acquired and saved by use of an SIM software routine (supplied by Hewlett-Packard) with a mass dwell time of 100 ms for each ion. Peak area integration and calculation were carried out by use of a software routine written by the author. The mass spectrometer was calibrated to perfluorotributylamine by use of an AUTOTUNE software routine supplied by Hewlett-Packard.

Gas Chromatography—A Perkin-Elmer Sigma 3 equipped with a 1.8-m (6-ft) by 6.35-mm (¼-in.) outside diameter glass column packed with 3% OV-17 on Chromosorb WHP, 100-120 mesh, was used. The nitrogen flow was 30 mL/min. The injector, detector, and column temperatures were 280, 280, and 270°C, respectively. The gas chromatograph was coupled to a Spectra Physics SP 4000 data processor.

High Performance Liquid Chromatography—A Hewlett-Packard 1084 B System equipped with a 25-cm by 4.6-mm inside diameter stainless steel column packed with 10 μm of octylsilane-bonded silica was used. The mobile phase consisted of 55% acetonitrile and 45% aqueous buffer containing 0.75 g of ammonia acetate per 100 mL. The flow of the mobile phase was set at 1 mL/min, and the detector was set at 280 nm light source.

Synthesis of Deuterated Heroin

Morphine (British Pharmacopeia [BP]) (0.4 g) and acetic anhydride-d_6 (5 mL) were placed in a 10-mL stoppered tube and heated at 80°C for 2 h. The resulting dark solution was evaporated to dryness under a stream of nitrogen. About 5 mL of distilled water was then added to the gel-like residue. The resulting pinkish solution was poured into a 25-mL beaker cooled in an ice bath, and 2*N* sodium carbonate was added by drops with stirring until the solution became basic. The precipitate was filtered and washed twice with distilled water and then dried in an oven overnight at 105°C.

Reagents

All reagents and chemicals used were analytical reagent grade. The HPLC grade acetonitrile and acetic anhydride-d_6 were obtained from Merck. Diamorphine (BP) was obtained from MacFarlan Smith, Ltd., England. Morphine (BP) was obtained from Singapore Pharmaceutical Department.

Preparation of Standard Solutions

Diamorphine standard solutions of 0.5, 1.0, 1.5, and 2.0 mg of base per millilitre were prepared by dissolving the appropriate amount of diamorphine (BP) in 10 mL of chloroform.

The deuterated diamorphine solution was prepared by dissolving 100 mg of the compound in 100 mL of chloroform.

Preparation of Sample Solutions

Samples were ground into fine powder and homogenized. About 100 to 200 mg of the powder was dissolved in 50 mL of chloroform/methanol (9:1). Five millilitres of the sample solution was mixed with 5 mL of deuterated diamorphine standard solution (or internal standard solutions for GC and HPLC).

Results and Discussion

The Hewlett-Packard 5985 B GS/MS system consists of a quadrupole mass analyzer which is well suited for SIM because selected ions from any region of the mass range can be monitored without altering optimum conditions in the ion source or mass analyzer. Further, the parameters (superimposed radio frequency and direct current fields) that control the

mass scale can be changed rapidly with good response and with no drift throughout the mass range for several hours [6]. In addition, SIM monitors only ions characteristic of the compound of interest. A peak in the characteristic ion chromatogram at the expected retention time is good evidence of the presence of the compound of interest. If two or more characteristic ions were monitored simultaneously and all the selected ion peaks were observed in the expected abundance ratio and at the expected retention time, the presence of the compound of interest should be highly evident. However, it is always necessary to generate a total electron impact mass spectrum to identify the compound of interest. Since SIM spends essentially all of the analysis time monitoring the selected ions instead of scanning a large mass range, much more sampling data are collected for each selected ion; therefore, quantitatively, more reliable results are obtained. The mass dwell time of 100 ms was chosen so that more cycles (about 75) were scanned over the heroin peak with good signal-to-noise ratio to minimize sampling error [7].

SIM is primarily a quantitative GC technique using the mass spectrometer as a detector. Thus, all facets of good GC technique apply. An internal standard is usually employed with a stable isotope-labeled compound whose chemical properties can closely match the compound of interest [8]. Under most GC conditions, the compound and its deuterated analog will co-chromatograph with the distinction that the molecular ion in the "fragmentographic" pattern will differ in mass by the number of deuterons substituted [9,10]. For better mass discrimination and determination, a resultant mass increase of at least 2 AMU is recommended.

Deuterated diamorphine synthesized from morphine and acetic anhydride-d_6 have two deuterated acetyl groups that offer an increase of 6 AMU. Figure 1 shows the mass spectra of diamorphine and deuterated diamorphine in the mass range of 250 to 380 AMU. It is seen that ions *m/e* 369 and 327 of diamorphine and 375 and 331 of deuterated diamorphine are all well isolated and free from interference. It was therefore decided that ions *m/e* 375 and 331 were to be used as internal standards and ions *m/e* 369 and 327 were to be quantitated

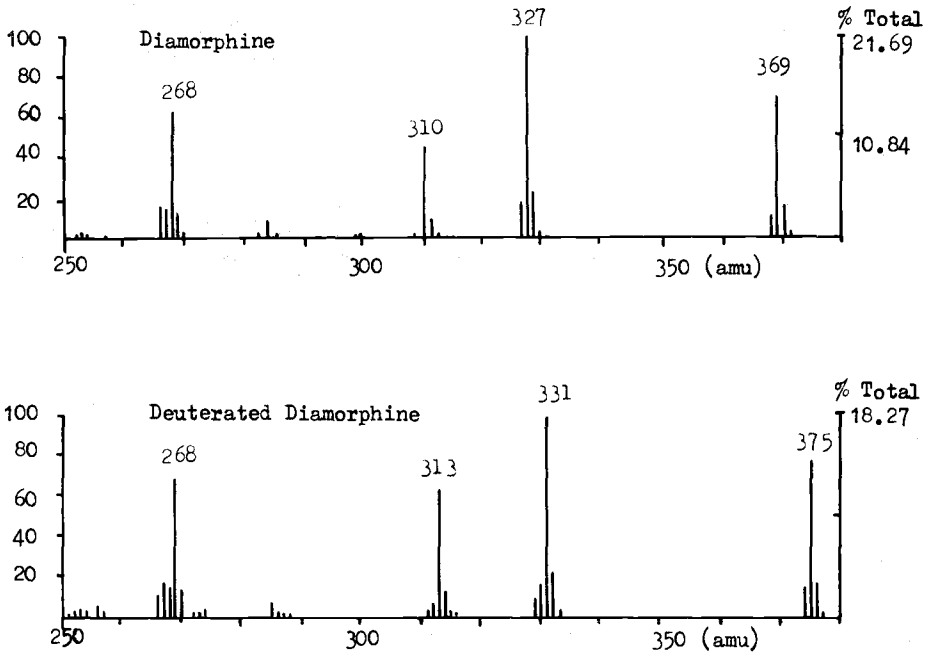

FIG. 1—*Mass spectra of diamorphine and deuterated diamorphine.*

and calculated as diamorphine. Figure 2 shows the real-time ion chromatogram of the four selected ions. Although diamorphine chromatographed slightly slower (about 6 s) than deuterated diamorphine, that delay was not expected to contribute any significant sampling error to the final results.

Figure 3 shows the calibration curves for both *m/e* 375/369 and *m/e* 331/327. Both were linear within the concentrations studied. To estimate the accuracy and precision of the method, five samples of different weights were taken from an illicit heroin sample and analyzed. Table 1 lists the statistical results together with results derived from GC and HPLC. In the course of analysis, it was found that the mass spectrometer must be recalibrated to perfluorotributylamine after every two analyses in order to get more reproducible results. Figures 4 and 5 show the HPLC and GC chromatograms, respectively, at the conditions stated in Experimental Procedures.

Table 2 lists some results obtained from exhibits submitted for analysis by various law enforcement agencies. GC was routinely used to quantitate illicit heroin and the results are included. HPLC was recently set up and some of the results are also included in Table 2.

All the results indicate that SIM is at least as accurate and precise as both GC and HPLC and has one distinct advantage in that it selectively quantitates diamorphine within that chromatographic peak. The choice of quantitating two characteristic ions further enhances the absolute accuracy of the diamorphine content. Should the two ions *m/e* 369 and 327 give significantly different results, the entire analysis must be carefully evaluated to locate the source of error, whether experimental, instrumental, or sample contamination.

The agreement of results obtained from SIM, GC, and HPLC suggests that local illicit heroin samples can be satisfactorily chromatographed to isolate heroin for quantitation. In practice, heroin may be quantitated by any one of the three methods. In critical cases where legal penalties depend on the amount of diamorphine involved, all three methods should be used. If, within experimental error, identical quantitation of heroin is obtained by all three methods, no reasonable doubt exists to dispute the diamorphine content in the sample. It should be noted that whenever SIM is chosen as a quantitative technique, an electron impact

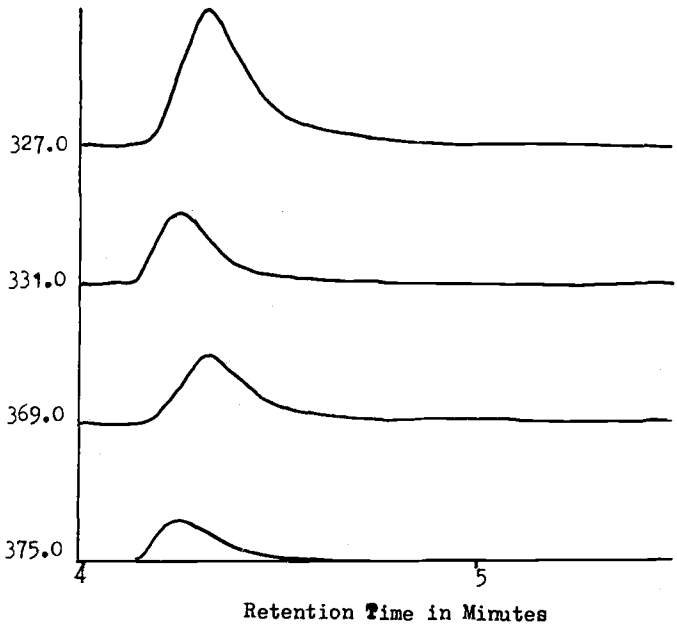

FIG. 2—*Real time ion chromatograms.*

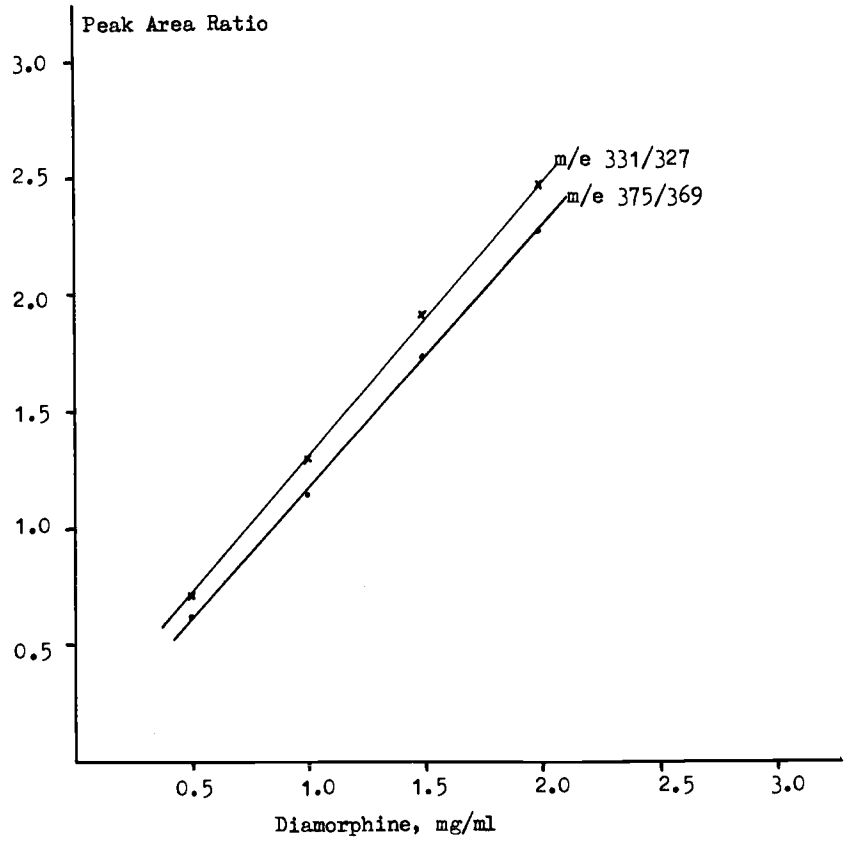

FIG. 3—*Calibration curves.*

TABLE 1—*Diamorphine percentages: results from five samples of different weights.*[a]

Sample Weight, mg	SIM		GC	HPLC
	m/e 375/369	*m/e* 331/327		
74.6	39.4	40.1	39.4	38.8
100.7	39.2	40.5	38.9	39.3
123.6	39.7	41.1	40.3	39.5
153.2	39.8	40.8	40.0	38.7
174.2	40.3	41.3	39.7	38.6
Mean	39.6	40.7	39.6	38.9
Standard deviation	0.42	0.47	0.54	0.39
Coefficient of variation, %	1.06	1.17	1.36	1.01

[a]All results were the averages of three injections.

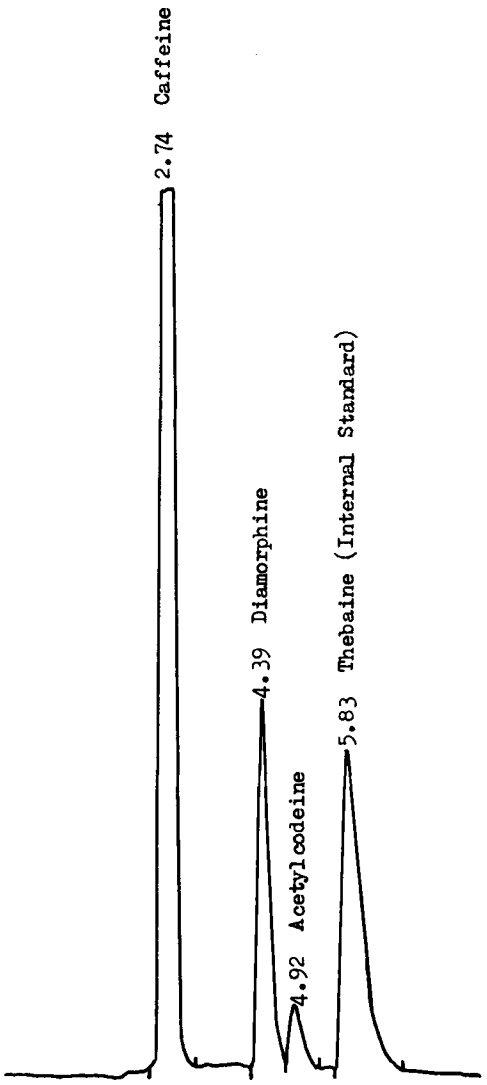

FIG. 4—*High performance liquid chromatogram.*

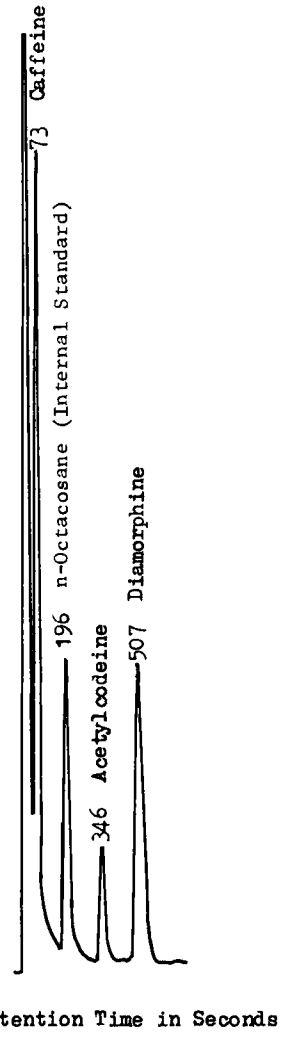

FIG. 5—*Gas chromatogram.*

mass spectrum must be obtained to ascertain the identity of diamorphine because acetyldihydromorphinoneenol acetate "pseudoheroin" also gives ions at *m/e* 369 and 327 but with a significantly different total electron impact fragmentation pattern [*11*].

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TABLE 2—*Diamorphine percentages: results from twelve samples submitted for analysis by law enforcement agencies.*[a]

Sample	SIM		GC	HPLC
	m/e 375/369	*m/e* 331/327		
A	40.9	40.0	40.7	40.1
B	43.3	44.8	44.2	44.0
C	31.4	32.2	32.9	32.1
D	17.7	17.9	17.1	...
E	67.4	67.6	69.5	...
F	68.7	67.7	69.1	...
G	80.3	80.9	81.8	...
H	28.7	29.7	30.1	...
I	75.6	76.1	77.8	...
J	75.9	74.7	75.5	...
K	40.0	39.3	39.4	...
L	38.6	38.1	39.7	...

[a]All results were the averages of three injections. Those without HPLC data were analyzed prior to HPLC setup.

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