A. F. Hernández,[1] Ph.D.; A. Pla,[1] Ph.D.; J. Moliz,[2] B.Sc.; F. Gil,[1] M.D.; M. C. Gonzalvo,[1] M.D.; and E. Villanueva,[1] Ph.D.

Application of the Combined Use of HPLC/Diode Array Detection and Capillary GC/Nitrogen Phosphorus Detection for the Rapid Analysis of Illicit Heroin and Cocaine Samples

REFERENCE: Hernández, A. F., Pla, A., Moliz, J., Gil, F., Gonzalvo, M. C., and Villanueva, E., **"Application of the Combined Use of HPLC/Diode Array Detection and Capillary GC/Nitrogen Phosphorus Detection for the Rapid Analysis of Illicit Heroin and Cocaine Samples,"** *Journal of Forensic Sciences*, JFSCA, Vol. 37, No. 5, September 1992, pp. 1276–1282.

ABSTRACT: A method using a combination of high-performance liquid chromatography and diode array detection (HPLC/DAD) with a methanol/perchlorate buffer pH 2.5 mobile phase and reverse phase C-18 column and capillary gas chromatography with nitrogen phosphorus detection (GC/NPD) has been developed for the rapid, sensitive and accurate analysis of illicit samples of heroin or cocaine. Retention times and UV-spectra were used for the identification of various adulterants and synthetic by-products in street samples. This method is compared with gas-chromatography/mass spectrometry (GC/MS). Forty street samples were analyzed by both methods. It is concluded that the combined use of HPLC/DAD and GC/NPD as a routine analytical procedure for the analysis of illicit samples could be a good alternative to GC/MS for forensic purposes. It is due to their high accessibility and the possibility to perform simultaneously qualitative and quantitative analysis.

KEYWORDS: toxicology, illicit samples, adulterants, heroin, cocaine, TLC, HPLC/DAD, GC/NPD, GC/MS

The analysis of abuse drugs, adulterants and impurities in street samples has been deeply studied from several points of view with different techniques, including simple color test, thin-layer chromatography, gas chromatography and liquid chromatography. Nevertheless, GC/MS is the method of choice for the unequivocal identification of heroin and cocaine.

Various high-performance liquid chromatographic (HPLC) systems have been reported for heroin or cocaine analysis, including ion exchange, adsorption, reverse phase, and reverse phase ion-pair chromatography. The best results are achieved when the last system is used [*1*]. Billiet et al. [2] reported a HPLC system with photodiode array detection

Received for publication 30 Aug. 1991; revised manuscript received 25 Jan. 1992; accepted for publication 28 Jan. 1992.

[1]Toxicologist, chief toxicologist, toxicologist, Clinical biochemist and chairman, respectively. Dept. of Legal Medicine and Toxicology Service, Faculty of Medicine, University of Granada, Granada, Spain.

[2]Chemist, Laboratory for Instrumental Analysis, University of Granada, Spain.

using a coupled alumina and C18 column, which is suitable for the separation of both adulterants and impurities in illicit heroin samples.

Different HPLC techniques for the adulterated cocaine samples have been reported, using UV-detection [3], electrochemical detection [4] and photodiode array detection, which permits the separation of carboxilic acids and mono-protic and di-protic amines [5].

Capillary gas-chromatography with nitrogen phosphorus detection has so far received comparatively little attention in illicit drug analysis. Only a few methods have been described for the identification of adulterant and impurities present in illicit heroin samples [6–8]. Those studies were carried out on standard mixtures of drugs or in a representative illicit heroin sample. However, we have not found any report of GC/NPD analysis of cocaine.

The purpose of this work was to establish a suitable method for the routine analysis of illicit heroin and cocaine samples in forensic laboratories. We present here a rapid, highly sensitive, specific and accurate method for the quantitation of heroin and cocaine as well as identification of adulterants and impurities in illicit street samples, using a combination of HPLC/DAD and GC/NPD. It is applicable to complex mixtures containing both abuse drugs, even at low levels.

This method was used for the analysis of 40 illicit street samples seized during 1989 and 1990. Samples were previously studied by thin-layer chromatography (TLC, ToxiLab^R system) and GC/MS.

Materials and Methods

Chemicals

HPLC grade methanol was obtained from Merck (Darmstadt, Germany). Other reagents were of analytical grade. Alkaloids were obtained from Merck (Darmstadt, Germany). Heroin was supplied by Dirección General de Farmacia (Madrid, Spain) and other drugs were of pharmaceutical grade and of different origins.

Samples

Forty street samples seized by the police in Granada during 1988 and 1989 were studied. Firstly, samples were analyzed by TLC and GC/MS.

TLC

For the TLC analysis the ToxiLab^R system (Analytical System, Division of Marion laboratories, Inc, Laguna Hills, CA, USA) was used. An amount of 2 to 3 mg of the sample was dissolved in 5 mL of distilled water. This solution was used for the extraction and further analysis according to Luna et al. [9].

GC/MS

A gas-chromatograph Hewlett-Packard Model 5890, equipped with a mass spectrometer Hewlett-Packard Model 5988 M was used. A 50 m × 0.2 mm SE-30 0.33 μm capillary column was used for the analysis. The program temperature was 80 to 320°C (initial 80°C, 1 min; ramp temperature 10°C/min; final temperature 320°C, 5 min), the injector and detector temperatures were 270 and 350°C, respectively. The helium carrier gas flow rate was at 1 mL/min. Samples were prepared by dissolving 10 mg of sample in 0.2 mL of methanol, and 0.2 μl were injected in a splitless mode.

HPLC

The HPLC chromatographic system consisted of a binary pump LC-250 Perkin Elmer, with a Diode Array Detector (LC-235 Perkin Elmer) and an integrator-registrator (LCI-100, Perkin Elmer) connected to a computer Epson PC AX2 for storage and manipulation of spectra. A reverse phase column (Pecosil HS/5 C-18, 0.46 × 12.5 cm, Perkin Elmer) was used. The mobile phase consisted of methanol and a perchlorate solution adjusted at pH 2.5 (modification of the method of Preskorn et al., [*10*]). The perchlorate solution was prepared by mixing 0.005 M perchloric acid with 0.045 M sodium perchlorate (9:1). The solvent was degased by filtering through organic:aqueous filter paper (Millipore FH VP 04700) primed with methanol. A linear gradient from 25 to 45% of methanol increasing 1%/min was used. After 15 min it was held at 45% for 5 min. Total run time was 20 min. The flow rate was 1.0 mL/min. The eluant was monitored at 230 nm. Samples were prepared at a final concentration of 0.25 mg/mL in methanol and 10 μl were injected.

GC/NPD

A Perkin Elmer 8310 gas chromatograph equipped with nitrogen phosphorus detector was used. A 15 m × 0.53 mm fused silica, 0.5 μm film, SPB 1 wide bore capillary column (a dimethylpolysiloxane stationary phase) was also used. The injector and detector temperatures were set at 270 and 300°C, respectively. The temperature program was started at 170°C, increasing 10°C/min to 270°C. This temperature was held for 5 min. A split-splitless capillary inlet system was operated in the split mode (1:25). Helium was used as carrier gas (1 mL/min). Samples were prepared at a final concentration of 1 mg/mL in methanol and 1 μl was injected.

Quantitation

The quantitative determination was performed on the basis of the peak areas using the external standard method. For all compounds, a linear relationship between the peak areas and the concentration was observed.

The coefficient of variation of the HPLC analysis of heroin and cocaine (50 μg/mL) was 4.7 and 3.4% (n = 5), respectively. When both drugs were assayed by GC/NPD (1 mg/mL), the coefficient of variation was 5.2 and 4.1% (n = 5), respectively.

Results

Table 1 shows the compounds detected by HPLC/DAD as well as their concentration, expressed in percentage of original powder.

Of 40 street samples, 75% contained heroin and 25% cocaine. Only two of them contained both heroin and cocaine. The concentration of heroin ranged from 1 to 39%; greater concentrations of cocaine (12 to 85%) were found in the samples containing that substance. Adulterants were detected in 67% of the samples containing heroin and 60% of samples containing cocaine. The most common adulterants in the samples containing heroin were caffeine (50%), procaine (47%), phenobarbital (13%) and acetaminophen (7%), while benzocaine was the most common adulterant (40%) in the samples containing cocaine, followed by lidocaine (20%).

All the heroin samples contained acetylmorphine. In 23 samples its concentration was less than 10%. The highest concentration of acetylmorphine found was 24%. Morphine was present in approximately ¼ of the samples, in concentrations of up to 4.3% of the sample weight, whereas codeine was found in only one sample and in concentration of 2%. Papaverine and noscapine were detected in 60% of the illicit heroin samples and

TABLE 1—*Composition of 40 illicit samples.*

Illicit Sample	N[a]	Purity[b] (Range)
Heroin	30	1–39%
Adulterants		
Caffeine	15	0.5–24%
Procaine	13	0.5–18%
Phenobarbital	4	0.5–2%
Acetaminophen	2	7–13%
Impurities		
AcetylMorphine	30	0.8–24%
Noscapine	18	3–41%
Papaverine	18	0.1–2.7%
Morphine	7	1.2–4.3%
Codeine	1	2%
Cocaine	12	12–85%
Adulterants		
Benzocaine	4	0.8–10%
Lidocaine	2	4.8–4.9%
Mepivacaine	1	N.D.
Impurities		
Benzoylecgonine	5	N.D.

[a]Number of samples where the compound was detected.
[b]Content of compound as percentage of sample weight.
N.D.: Not determined.

always both alkaloids were present. Concentrations of papaverine and noscapine of up to 2.7 and 41%, respectively, were detected.

Good agreement was obtained between the adulterants and impurities determined by HPLC/DAD, GC/NPD, and GC/MS. Larger discrepancies were obtained when TLC is compared with other methods. Figure 1 shows typical chromatograms of an illicit sample analyzed by different chromatographic methods.

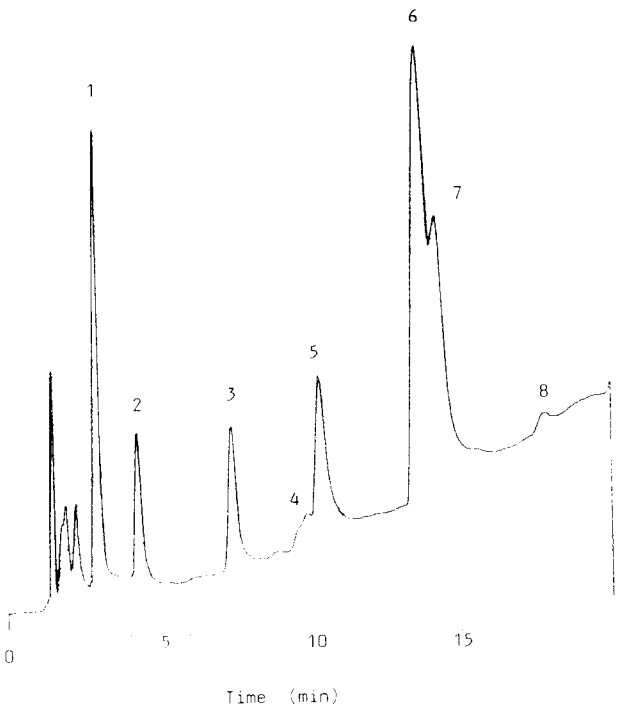
Discussion

The purpose of this work has been to check the efficiency of HPLC/DAD and GC/NPD with respect to other systems (TLC-ToxiLab[R] and GC/MS) in order to be used routinely in the forensic laboratory for the quantitation and identification of heroin and cocaine, as well as adulterants and impurities in illicit samples.

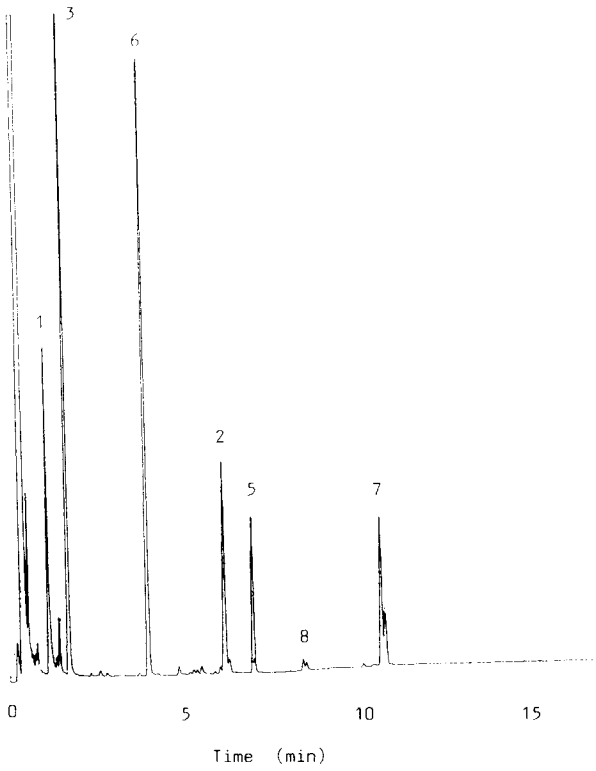
TLC has been previously used in our laboratory as a system for the screening of illicit heroin samples [*9*]. With this method, the most common adulterants are detected, but only permits a qualitative assay of samples, except when a densitometric UV direct measurement is used [*11*]. Furthermore, it is time-consuming, and some substances can be erroneously identified since they have similar migration and color characteristics. An additional problem is the lack of sensitivity for some adulterants that could be present in very low concentrations.

GC/MS is undoubtedly the choice method for such a purpose and served as the reference method. It was able to resolve acetylcodeine from acetylmorphine, and the whole substances (like impurities, synthetic by-products and adulterants) were detected. Nevertheless, GC/MS usually is not available for small laboratories, and presents other additional problems. In our assay conditions, noscapine undergoes thermal fragmentation providing two peaks, one of them coeluted with caffeine.

HPLC/DAD offers an interesting alternative to the other methods. It is sensitive, rapid, specific and accurate for quantitation of heroin or cocaine and the identification



A



B

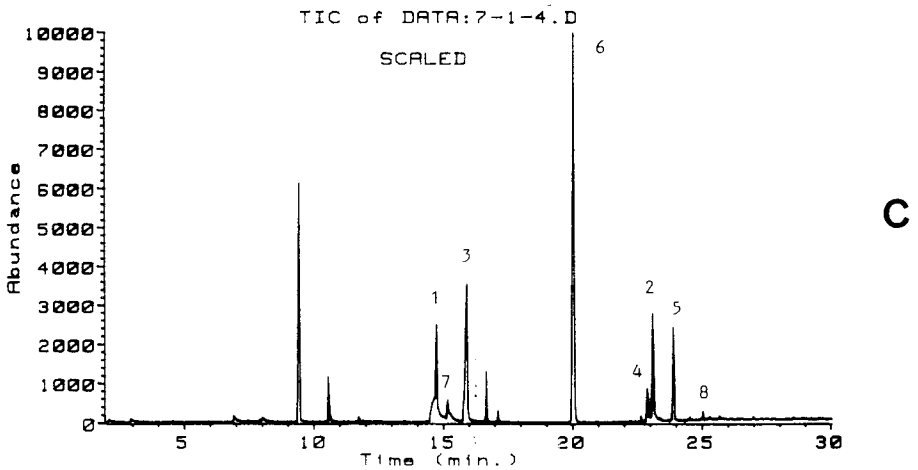


FIG. 1—*Chromatograms of a representative illicit sample containing: (1) Acetaminophen; (2) Acetylmorphine; (3) Caffeine; (4) Acetylcodeine; (5) Heroin; (6) Cocaine; (7) Noscapine; (8) Papaverine. Conditions for HPLC/DAD (A), GC/NPD (B) and GC/MS (C) are described in text.*

of adulterants and impurities. The HPLC method used was capable to detect the same number of substances than GC/MS (see Table 1 and Fig. 1 as examples) with the exception of acetylcodeine that coeluted with heroin. Because the amount of heroin is usually much greater than that of acetylcodeine, the effect of the interference is generally small.

The use of a linear gradient from 25 to 45% of methanol permeted a good resolution of almost all compounds with a short time of analysis (20 min).

On the other hand, the use of DAD permits the identification of components based in the retention time and the characteristic UV-spectrum of each component. This system is easier than the absorbance ratios at two different wavelengths. A further specific possibility is using a derivative absorption spectroscopic system in order to identify different substances with a very similar spectra.

All adulterants identified by HPLC/DAD and GC/MS were also detected with GC/NPD. So, the simultaneous assay of a sample by HPLC/DAD and GC/NPD offers the possibility to achieve the identification and confirmation of different components in illicit street samples, which is an important aspect from a forensic point of view. Furthermore, the quantitation of heroin and cocaine by both techniques showed a good correlation (r = 0.912 and 0.975, respectively).

It is concluded that the combined use of HPLC/DAD and GC/NPD as a routine analytical procedure for the analysis of illicit samples could be a good alternative to GC/MS for forensic purposes. It is due to their high accessibility and the possibility to perform simultaneously qualitative and quantitative analysis.

References

[1] Lurie, I. S., Sottolano, S. M., and Blasot, S., "High Performance Chromatographic Analysis of Heroin by Reverse Phase Ion-Pair Chromatography, *Journal of Forensic Sciences*, Vol. 27, No. 3, July 1982, pp. 519–526.

[2] Billiet, H. A., Wolters, R., De Galan, L., and Huizer, H., "Separation and Identification of Illicit Heroin Samples by Liquid Chromatography Using an Alumina and C18 Coupled Column System and Photodiode Array Detection," *Journal of Chromatography*, Vol. 368, No. 2, October 1986, pp. 351–361.

[3] Gill, R., Abbott, R. W., and Moffat, A. C., "High-Performance Liquid Chromatography Systems for the Separation of Local Anesthetic Drugs with Applicability to the Analysis of Illicit Cocaine Samples," *Journal of Chromatography*, Vol. 301, 1984, pp. 155–163.

[4] Selavka, C. M., Krull, I. S., and Lurie, I. S., "An Improved Method for the Rapid Screening of Illicit Cocaine Preparations Using High-Performance Liquid Chromatography with Electrochemical Detection," *Forensic Sciences International*, Vol. 31, No. 2, June 1986, pp. 103–117.

[5] Lurie, I. S., Moore, J. M., Cooper, D. A., and Kram, T. C., "Analysis of Manufacturing By-Products and Impurities in Illicit Cocaine via High-Performance Liquid Chromatography and Photodiode Array Detection," *Journal of Chromatography*, Vol. 405, September 1987, pp. 273–281.

[6] Machata, G. and Vycudilik, W., "Gas Chromatographic Analysis of Illicit Heroin Samples," *Journal of Analytical Toxicology*, Vol. 4, 1980, pp. 318–321.

[7] Demedts, P., Van den Heede, M., Van den Verren, J., and Heyndrickx, A., "Combined Use of Fused Silica Capillary Columns and Nitrogen-Phosphorus Detection for the Toxicological Analysis of Illicit Heroin Samples," *Journal of Analytical Toxicology*, Vol. 6, 1982, pp. 30–33.

[8] Alm, S., Hohnson, S., Karlsson, H., and Sundholm, E. G., "Simultaneous Gas Chromatographic Analysis of Drugs of Abuse on Two Fused-Silica Columns of Different Polarities," *Journal of Chromatography*, Vol. 254, 1983, pp. 179–186.

[9] Luna, A., Pla, A., Martos, M., and Villanueva, E., "Adulterants in Illicit Heroin Samples," *Revista de Toxicologia*, Vol. 1, 1984, pp. 125–133.

[10] Preskorn, S. H., Leonard, K., and Hignite, C., "Liquid Chromatography of Amytriptiline and Related Tricyclic Compounds," *Journal of Chromatography*, Vol. 197, 1980, pp. 246–250.

[11] Della Casa, E. and Martone, G., "A Quantitative Densitometric Determination of Heroin and Cocaine Samples by High-Performance Thin-Layer Chromatography," *Forensic Science International*, Vol. 32, No. 2, October 1986, pp. 117–120.

Address requests for reprints or additional information to
Antonio F. Hernández, Ph.D.
Department of Legal Medicine and Toxicology Service
Avda. de Madrid, 11
18071-Granada, Spain