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Analytical Studies on Illicit Heroin
II. Comparison of Samples

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ABSTRACT: A rapid method for the comparative analysis of illicit heroin samples has been developed. It is based on high pressure liquid chromatography using an ultraviolet and a fluorimetric detector simultaneously. The two detectors give so much information that reliable conclusions can be made.

KEYWORDS: toxicology, heroin, chemical analysis, chromatographic analysis

In investigating illicit heroin samples, it is sometimes necessary not only to perform qualitative and quantitative analysis of the samples, but also to ascertain whether or not the samples are identical. When evidence against a heroin dealer must be provided, the last point may be particularly important. In such cases the statements of heroin users can be supported by chemical analysis, which can show that the samples seized from the users and the dealers are identical. Chemical analysis may also provide evidence in cases in which heroin distribution chains are being investigated.

Several analytical methods can be used for the comparison of heroin samples. Qualitative differences in such samples can sometimes be observed by means of thin layer chromatography (TLC). However, when this method does not give unambiguous results, quantitative analysis of heroin and the various main impurities present in the samples must be performed. The pattern of the impurities may be extremely valuable for comparisons of heroin samples.

Qualitative analysis of impurities in several types of drugs by gas liquid chromatography (GLC) has been described by Strömberg [*1*] and Strömberg and Maehly [*2*]. The same technique is well suited for comparison of illicit heroin samples.

At the moment most of the illicit heroin samples seized in the Netherlands originate from the Middle East. These samples are characterized by a relatively high content of noscapine and papaverine. For comparative analysis of such heroin samples we developed a high performance liquid chromatography (HPLC) procedure, which is described in the present paper.

By TLC it was observed that Middle Eastern heroin samples contained a number of fluorescent components. Therefore the HPLC was performed using both an ultraviolet and a fluorimetric detector. In that way information valuable for discriminating among a series of

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heroin samples could be obtained. The method described is rapid and only small amounts of the sample are needed (20 mg).

Experimental Procedure

Materials

Twenty-five illicit heroin samples seized from the Dutch black market were used. Commercial samples of heroin, noscapine, and papaverine hydrochlorides were used as references. Acetylcodeine hydrochloride was obtained as a gift from the firm Diosynth (Apeldoorn, The Netherlands). Acetylthebaol, thebaol, and 3,6-dimethoxyphenantrene-4,5-epoxide were kindly supplied by Dr. H. G. Theuns (Department of Organic Chemistry, State University of Utrecht, The Netherlands).

Apparatus and Methods

The analyses were carried out on an SP 8000 liquid chromatograph (Spectra Physics, San Jose, CA) equipped with an ultraviolet detector (wavelength 227 nm) and a fluorimetric detector (excitation wavelength 260 nm and emission wavelength 400 nm). The stainless steel column (250 mm long and 4.6 mm inside diameter) was filled with Lichrosorb Si-60-7 (Merck, Darmstadt, West Germany). The mobile phase consisted of hexane-dichloromethane-methanol (72:20:5); the methanol contained 0.75% v/v diethylamine. The flow rate was 2.0 mL/min.

Sample Preparation

Ten millilitres of the solvent mixture used as a mobile phase was added to 20.0 mg of the heroin sample. The resulting mixture was placed for 5 min in an ultrasonic bath to dissolve.

Sample Injection

Of the solutions obtained—or the supernatants, if the sample was not completely dissolved—10-μL samples were injected into the chromatograph by a loop injection system.

Quantitative Determination

The quantitative determinations were performed on the basis of the peak areas using the external standard method. For all compounds analyzed a linear relationship between the peak areas and the amount of substance was observed.

Results and Discussion

Twenty-five uncut heroin samples of the so-called Middle Eastern type were analyzed by HPLC using ultraviolet and fluorimetric detection. Most of the samples contained heroin as base.

Ultraviolet Detection

To obtain sufficient sensitivity for the determination of noscapine and papaverine, the 227-nm wavelength was used. A typical chromatogram is given in Fig. 1. The computed percentages of heroin, acetylcodeine, noscapine, and papaverine in the analyzed samples are

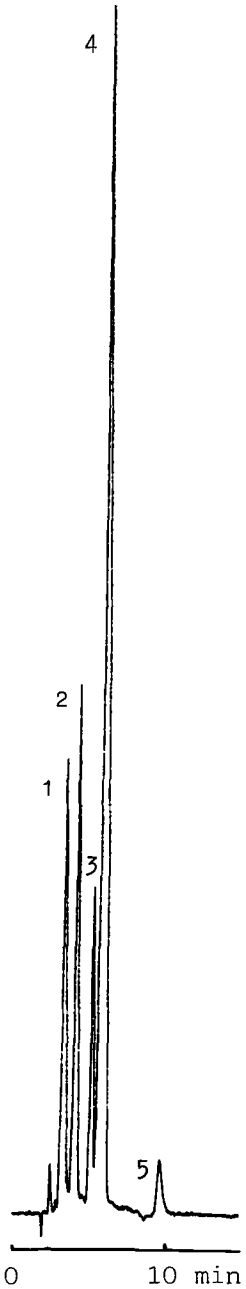

FIG. 1—*Typical chromatogram obtained for an illicit heroin sample by HPLC and ultraviolet detection. 1 = noscapine, 2 = papaverine, 3 = acetylcodeine, 4 = heroin, and 5 = O^6-monoacetylmorphine.*

given in Table 1. The relative standard deviations, computed by weighing, dissolving, and analyzing an average heroin sample ten times, are given in Table 2.

Heroin—In the uncut samples the heroin content varied within a narrow range, from 64 to 89% (calculated as hydrochloride). However, because the standard deviation for the heroin determination was only 1.5% the range was broad enough—20 times the standard deviation—to make some discrimination on the basis of the heroin content.

Acetylcodeine—The content of acetylcodeine—calculated as hydrochloride—varied from 4.3 to 7.4%, with an average of 5.7%. Since it was observed that the ratio between the amounts of heroin and acetylcodeine was rather constant (1:12 to 1:16), the content of acetylcodeine in the heroin samples was of minor value for comparison purposes.

Noscapine—The variation in the noscapine content was large (0.7 to 8.5%). Therefore, the content of noscapine was useful for discriminating among the illicit heroin samples.

Papaverine—The content of papaverine varied from 1.2 to 2.5%. Papaverine might thus be a useful compound for discriminating among the heroin samples. No correlation between the contents of noscapine and papaverine in the samples was observed.

The varying amounts of the four components in the heroin samples analyzed (heroin, acetylcodeine, noscapine, and papaverine) permitted discrimination among a number of the samples. Assuming that samples were considered to be not identical as soon as the data obtained for one of the four components differed by more than six times the standard deviation

TABLE 1—*Data obtained by HPLC and ultraviolet detection for 25 illicit heroin samples.*

Sample	Noscapine, %	Papaverine, %	Acetylcodeine, %	Heroin, %
1	8.5	1.4	5.9	85
2	5.8	2.1	6.7	83
3	2.9	1.9	6.9	84
4	2.0	1.3	5.0	78
5	2.5	1.4	5.8	77
6	2.4	1.4	5.7	74
7	7.1	2.2	4.7	77
8	0.7	1.2	5.6	76
9	0.9	2.1	7.2	87
10	0.8	1.3	5.5	73
11	0.7	1.7	6.1	83
12	2.0	1.5	5.3	73
13	0.9	1.4	5.2	73
14	0.9	2.2	7.3	88
15	0.9	2.2	7.4	89
16	0.8	1.4	4.4	68
17	0.8	2.4	5.3	66
18	0.8	1.4	4.9	66
19	1.4	1.2	4.3	64
20	3.0	2.5	6.3	80
21	3.0	1.6	5.8	88
22	2.6	1.5	5.4	84
23	1.3	1.3	5.1	73
24	1.4	1.3	5.3	76
25	1.3	1.3	5.4	80

TABLE 2—*Relative standard deviations of compounds determined.*

Compound	Standard Deviation, %
Noscapine	4.0
Papaverine	3.5
Acetylcodeine	3.3
Heroin	1.5

for that component, eight unique samples were found; the other 17 samples could be divided into groups consisting of two to five samples each. Mainly because of the data obtained for the content of noscapine and papaverine, better discrimination among the heroin samples was achieved than in a previous study [*3*]. In order to achieve a still better discrimination the studies were extended to HPLC with fluorimetric detection.

Fluorimetric Detection

The excitation and emission wavelengths were experimentally found to be optimum at 260 and 400 nm, respectively. A typical chromatogram obtained under such conditions is given in Fig. 2. The main peaks are marked *A*, *B*, *C*, and *D*; minor peaks—detected in some cases—*a*, *b*, *c*, and *d*.

Peak *A* was identified as acetylthebaol, a compound that can be present in illicit heroin samples as a result of the acetylation of thebaine [*4,5*]. However, usually its amount is so low that the compound can not be observed by the ultraviolet detector. The maximum content of acetylthebaol in the heroin samples analyzed amounted to 1.6%; mostly 0.2 to 0.8% were found. No correlation between the acetylthebaol content and the levels of noscapine and papaverine was observed.

Peak *a* was identified as 3,6-dimethoxyphenanthrene-4,5-epoxide, and shoulder *b* as thebaol. The first compound might also originate from the acetylation of thebaine, whereas thebaol might be formed by hydrolysis of acetylthebaol [*4,5*].

So far the other peaks could not be identified. However, it was established that during the acetylation of thebaine not only acetylthebaol but also compounds with the same retention times as Peaks *B* and *C* were formed.

No peaks of heroin, acetylcodeine, noscapine, or papaverine could be observed in the chromatograms under the conditions used for fluorimetric detection.

The chromatograms obtained by HPLC and fluorimetric detection for 24 of the 25 heroin samples analyzed are shown in Fig. 3. To facilitate a comparison the chromatograms were grouped on the basis of the height of the first main peak, acetylthebaol. As can be seen, large variations in the amount of this compound were found. In samples where the amount of acetylthebaol was similar, discrimination was possible on the basis of the ratios between the Peaks A and B or C. In that way 18 unique samples, two pairs of similar samples (5 and 6/10 and 13) and one group consisting of three similar samples (9, 14, and 15) could be distinguished.

It was assumed that Samples 5 and 6; 10 and 13; and 9, 14, and 15 were identical, since they were also similar in color and content of heroin, acetylcodeine, noscapine, and papaverine. In addition, no differences could be found by using the GLC method described by Strömberg [*1*].

Thus HPLC and fluorimetric detection permitted sharp discrimination among the different heroin samples.

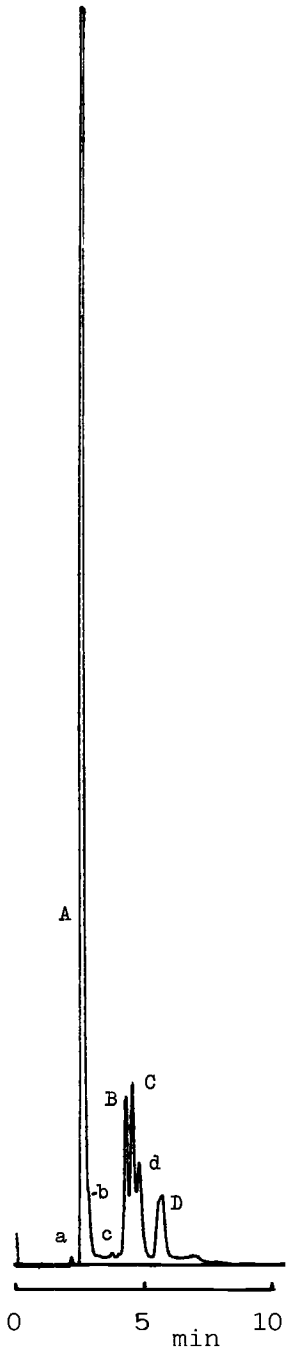

FIG. 2—*Typical chromatogram obtained for an illicit heroin sample by means of HPLC and fluorimetric detection.* A = *acetylthebaol,* a = *3,6-dimethoxyphenanthrene-4,5-epoxide, and* b = *thebaol. Other peaks are unknown.*

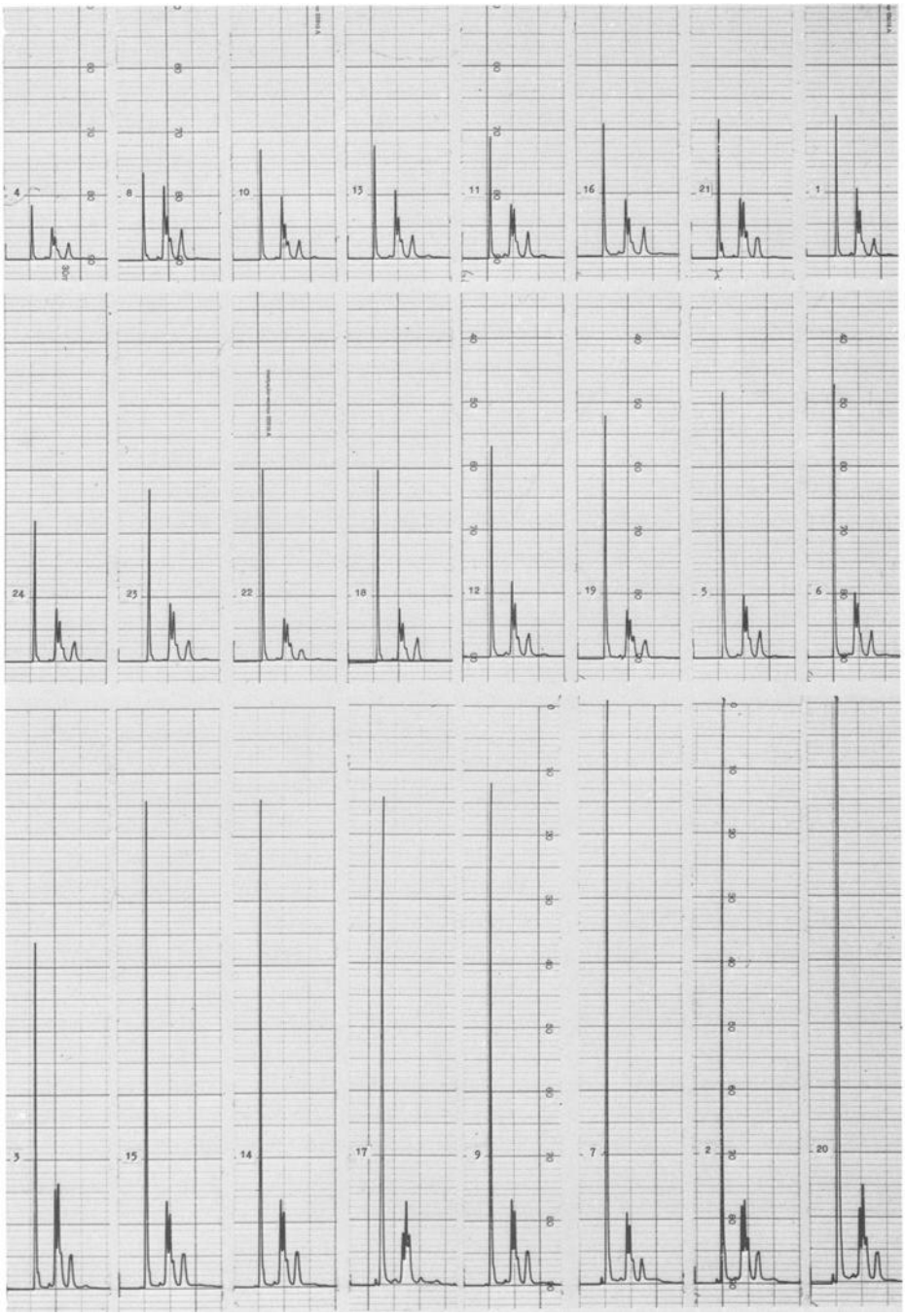

FIG. 3—*Chromatograms obtained for 24 illicit heroin samples by means of HPLC and fluorimetric detection.*

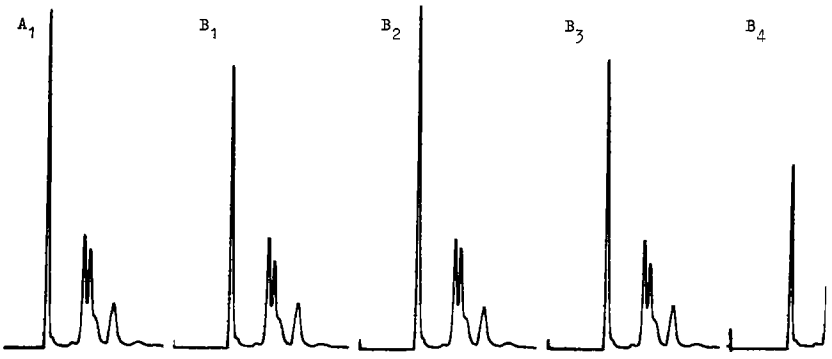

FIG. 4—*Chromatograms obtained by means of HPLC and fluorimetric detection in Case 1.*

TABLE 3—*Chemical analysis of heroin samples in Case 1.*

Sample	Noscapine, %	Papaverine, %	Acetylcodeine, %	Heroin, %	Color
A1	1.38	1.53	5.42	74.6	brown
B1	1.39	1.52	5.77	82.6	beige
B2	1.36	1.54	5.47	74.4	brown
B3	1.27	1.48	5.70	82.1	beige
B4	1.31	1.46	6.06	91.9	light brown

Generally, by comparing the appearance (color) of illicit heroin samples and the analytical data obtained by means of HPLC and ultraviolet detection as well as HPLC and fluorimetric detection, it is possible to establish whether or not samples are identical, as illustrated by a couple of examples.

Case 1

A suspect was in the possession of a sample of 100-g heroin (Sample A1). It was claimed that the sample originated from a dealer, in whose possession four samples of heroin were found (Sample B1, 100 g; B2, 154 g; B3, 102 g; and B4, 385 g). Table 3 shows the results obtained by chemical analysis using HPLC and ultraviolet detection.

The data gave rise to the supposition that Sample A1 corresponded with Sample B2, but they were not sufficient to guarantee that the samples were identical, because they fell within the range of "average" heroin samples. However, the chromatograms obtained by HPLC and fluorimetric detection (Fig. 4), further documented the similarity of Samples A1 and B2.

Case 2

A suspect was in the possession of 3-g heroin, and in his hotel room two further samples were found, each of 100-g heroin.

HPLC analysis showed that all three samples were mixtures of Middle Eastern heroin and dipyrone. Heroin cut with dipyrone is rarely found in the Dutch black market. Testing by HPLC and ultraviolet detection showed practically the same content of heroin, acetylco-

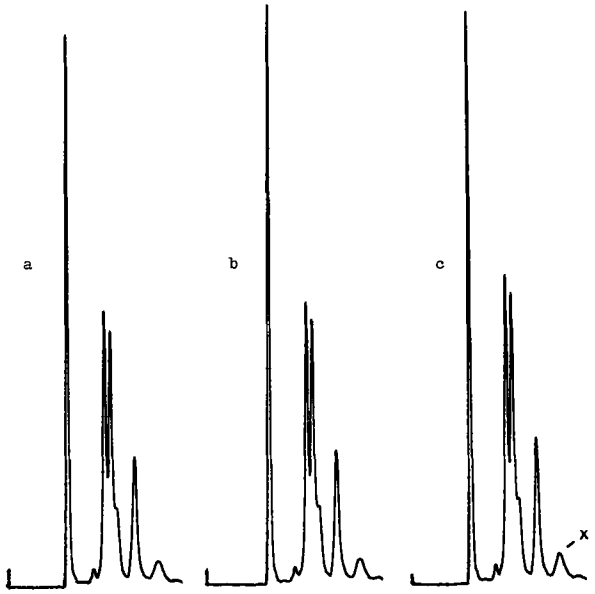

FIG. 5—*Chromatograms obtained by means of HPLC and fluorimetric detection in Case 2.*

deine, noscapine, and papaverine. The chromatograms obtained by HPLC and fluorimetric detection are shown in Fig. 5. Although dipyrone could not be observed by fluorimetric detection a small peak (*x*), possibly an impurity present in dipyrone, was found. On the basis of the results it was concluded that all three samples of heroin were identical.

Acknowledgments

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