Journal of Chromatography, 366 (1986) 363–372
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 802

ANALYSIS OF ILLICIT HEROIN

I. AN EFFECTIVE THIN-LAYER CHROMATOGRAPHIC SYSTEM FOR SEPARATING EIGHT OPIATES AND FIVE ADULTERANTS

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(First received June 4th, 1985; revised manuscript received May 12th, 1986)

SUMMARY

A thin-layer chromatographic (TLC) system with chloroform–*n*-hexane–triethylamine (9:9:4) as eluent that is capable of separating eight opiates and five potential adulterants, has been developed for the analysis of illicit heroin. The system was tested using illicit heroin samples and the results were confirmed by gas chromatography. The limit of detection is 0.1 μg. Thirty-five TLC systems reported in the literature for opiate analysis were classified according to their resolving power.

INTRODUCTION

Heroin (diacetylmorphine, DAM) is prepared by the diacetylation of morphine, an alkaloid of opium. Depending on the purity of the starting material (morphine base *vs.* raw opium) and the manufacturing process involved, the heroin produced may contain some other opium alkaloids such as paraverine, thebaine, noscapine and codeine (natural alkaloids) or acetylcodeine and 6-monoacetylmorphine (synthetic alkaloids resulting from the acetylation step). In addition to these eight opiates, heroin at street level could contain other interfering substances such as methadone, cocaine, caffeine, ephedrine and quinine. Thus information relating to the source, trafficking and distribution patterns of the illicit heroin trade could be deduced from a detailed analysis of the product.

Thin-layer chromatography (TLC) is the most popular screening method for opiates[1–15]. However, an assessment of published solvent systems carried out in this laboratory demonstrated their unsuitability for simultaneously identifying the above eight opiates and five adulterants as they were designed specifically to identify a selected number of opiates and adulterants.

In this paper we report a TLC solvent system that is capable of separating the above 13 components. The TLC system was tested on samples of illicit heroin and the results were confirmed by gas chromatography (GC). The GC data was used for grouping the samples according to their chemical composition.

0021-9673/86/$03.50

EXPERIMENTAL

The solvents used were of analytical-reagent grade and were not purified further. Opiates and other chemical standards were obtained from the United Nations Division on Narcotic Drugs, Vienna. Caffeine was purchased locally. Samples for TLC and GC were prepared in chloroform–methanol (9:1) solution.

TLC was performed on Merck pre-coated plates (20 × 20 cm, aluminium backed; silica gel 60 GF_{254}, 0.2 mm thickness) and developed in chloroform–*n*-hexane–triethylamine (9:9:4). The developed plates were examined under UV light (254 and 366 nm) and treated with spray reagents.

The GC analysis was carried out on a Hewlett-Packard HP 5880A gas chromatograph equipped with a flame ionization detector. The glass column (6 ft. × 0.2 mm I.D.) was packed with 3% OV-210 (unsilanized) on 100–120-mesh Chromosorb W. The column oven was temperature programmed from 190°C (15 min) to 270°C (5 min) at 8°C min^{-1}. The carrier gas was nitrogen (25 ml min^{-1}). The injector and detector temperatures were 270 and 300°C, respectively. Prior to GC analysis, the column was conditioned overnight at 270°C and 45 ml min^{-1}.

Two batches of illicit heroin samples were analysed. The first batch of four samples (A–D) was seized overseas whereas the second batch of 21 samples (Nos. 1–21) was seized locally (Malaysia).

RESULTS AND DISCUSSION

The chemical composition of a sample of street heroin can usually be classified into four groups:

(i) natural opium alkaloids (*e.g.*, morphine, codeine, noscapine, papaverine and thebaine), some of which may pass unchanged through the extraction, acetylation and purification procedure;

(ii) synthetic opium alkaloids [*e.g.*, 6-monoacetylmorphine (MAM), diacetylmorphine and acetylcodeine];

(iii) diluents (*e.g.*, caffeine, quinine, sugar and talc), which are added for bulk; and

(iv) adulterants (*e.g.*, methadone, cocaine and ephedrine), which produce added pharmacological effects.

The chemical composition of heroin intercepted in the illicit drug traffic and at consumer level varies substantially, depending on factors such as the geographical source, the manufacturing process used (extraction, acetylation and purification) and the distribution pattern (international and local). Thus a detailed chemical analyses of a sufficient number of samples would contribute towards providing data relating to their origin and distribution pattern. As a preliminary analytical method, a TLC system that is capable of resolving the components of an illicit heroin sample would contribute towards monitoring illicit opiate traffic.

The TLC systems reported in literature were evaluated for their suitability for analysing illicit heroin and are listed in Table I. These systems were assessed using a mixture of the eight opiates and five adulterants and were classified according to their ability to resolve the major opiates (without interference from the other components of the standard mixture) in the following manner: (i) urine (morphine and

TABLE I

ASSESSMENT OF PUBLISHED TLC SYSTEMS FOR OPIATE ANALYSIS

Abbreviations: Acet = acetone; amyOH = amyl alcohol; Bz = benzene; $CHCl_3$ = chloroform; cycHex = cyclohexane; Diox = dioxane; DEA = diethylamine; EtOAc = ethyl acetate; Et_2O = diethyl ether; EtOH = ethanol; H_2O = water; iPrOH = isopropanol; MeOH = methanol; MeCN = acetonitrile; nBu_2O = *n*-butyl ether; NH_3 = ammonia solution; nHex = *n*-hexane; Pyr = pyridine; t-amyDH = *tert.*-amyl alcohol; Tol = toluene. Numbers: 1 = morphine; 2 = 6-monoacetylmorphine; 3 = diacetylmorphine; 4 = codeine; 5 = acetylcodeine; 6 = noscapine; 7 = papaverine; 8 = thebaine; 9 = ephedrine; 10 = quinine; 11 = methadone; 12 = caffeine; 13 = cocaine.

Ref.	*Solvent system*	*Opiate and adulterants*	*Interfering substances**	*Category***
1	EtOAc–cycHex–Diox–MeOH–H_2O–NH_3 (50:50:10:10:1.5:0.5)	1, 2, 4, 6, 8 3 7	None 5, 12 13	(i)
	EtOAc–cycHex–MeOH–NH_3 (70:15:10:5)	1–5, 7, 8 6	None 11, 13	(i)
	EtOAc–cycHex–Diox–MeOH–H_2O–NH_3 (50:50:10:10:0.5:1.5)	1, 6, 7	2–5, 8, 9 None	–
	EtOAc–cycHex–NH_3–MeOH–H_2O (70:15:2:8:0.5)	1, 2, 4–7 3	None 8, 12	(i)
2	Bz–Diox–EtOH–NH_3 (50:40:5:5)	1, 4, 7, 8 2 3 6	None 10 5 13	(i)
	MeOH–NH_3 (100:1.5)	1 2 6	4 3, 5, 8, 11 7	–
	EtOH–HOAc–H_2O (6:3:1)	1 2, 4 5 6	3, 12 None 8 7, 10	–
3	Diox–$CHCl_3$–EtOAc–NH_3 (60:25:10:5)	1–5, 7, 8 6	None 13	(i)
	EtOAc–Bz–MeCN–HN_3 (25:30:40:5)	1–4, 6, 7 5	None 8, 12	(i)
	MeCN–$CHCl_3$–EtOAc–NH_3 (40:30:25:5)	1–4, 6, 7 5	None 8	(i)
	EtOAc–Bz–MeCN–NH_3 (50:30:15:5)	1–3, 6, 7 4 5	None 10 8, 12	–

(*Continued on p. 366*)

TABLE I (*continued*)

Ref.	*Solvent system*	*Opiate and adulterants*	*Interfering substances**	*Category***
	EtOAc–nBu_2O–NH_3 (60:35:5)	1 2 5 6, 7	4, 10 3, 8 12 None	–
4	EtOAc–iPrOH–NH_3 (40:30:3)	1, 4, 5, 6 2 7	None 3, 8, 10 11	(i)
5	$CHCl_3$–MeOH (9:1)	1 2 3 6 8	9 4, 11 5 7 None	–
	nBu_2O–Et_2O–DEA (45:45:10)	1, 3, 6 2 4 5	None 7 10 8	–
6	Bz–EtOAc–MeOH–NH_3 (80:20:6.5:0.1)	1 3 6, 7	2, 4, 9, 10 5, 8 None	–
	nBuOH–HOAc–H_2O (35:3:10)	1 2 3 6	4 8, 13 5 7	–
	nHex–EtOAc–NH_3 (60:40:0.1)	1 6	2–5, 7–13 None	–
	nBuOH–nBu_2O–NH_3 (25:70:2)	1 2 6, 7	4, 9 3, 5, 8, 12 None	–
7	EtOAc–MeOH–NH_3 (85:10:5)	1 2 4, 5, 6 7	9 3, 8 None 11	–
8	$CHCl_3$–cycHex–DEA (8:10:3)	1 2, 3, 5, 7 4 6	4, 12 None 12 8	–
	$CHCl_3$–EtOH–Acet–NH_3 (20:20:5:1)	1, 2, 4 3 6	None 5, 8, 12 7, 13	(i)
	$CHCl_3$–MeOH–DEA (16:3:1)	1 2 5 6	None 3, 4 12, 13 7, 8, 10, 12	–

TABLE I (*continued*)

Ref.	*Solvent system*	*Opiate and adulterants*	*Interfering substances**	*Category***
9	MeOH–nBuOH–Bz–H_2O (60:15:10:15)	1	4	–
		2	3, 5, 10, 13	
		6	7	
		8	None	
	EtOH–Pyr–Diox–H_2O (50:20:25:5)	1	11	–
		2	None	
		3	5, 10	
		4	8	
		6	7	
	t-amyOH–nBu_2O–H_2O (80:7:13)	1	2–5, 8, 13	–
		6, 7	None	
10	Tol–Acet–EtOH–NH_3 (20:20:3:1)	1, 4, 6, 7	None	(i)
		2	8, 12	
		3	5	
11	$CHCl_3$–Et_2O–MeOH–NH_3 (75:25:5:1)	1	9	–
		2–5	None	
		6	7, 13	
		8	11, 12	
12	nHex–$CHCl_3$–DEA (50:30:7)	1, 3–8	None	(ii)
		2	12	
13	Tol–Acet–EtOH–DEA (30:60:7:3)	1, 2, 4, 7	None	(i)
		3	5, 8, 12	
14	Tol–Acet–EtOH–DEA (45:45:7:3)	1, 2, 4, 7	None	(i)
		3	5, 8	
		6	11, 13	
	cycHex–Bz–DEA (70:25:10)	1	2, 10, 12	–
		3, 4	None	
		5	6, 8	
	$CHCl_3$–Acet (9:1)	1	2–5, 8–11	–
		6	None	
		7	13	
15	Tol–Acet–EtOH–NH_3 (40:40:6:2)	1, 4, 7	None	(i)
		2	10	
		3	5, 8	
		6	13	
	Tol–EtOAc–DEA (70:20:10)	1, 4, 6	None	(i)
		2	9, 12	
		3	5, 7, 8, 9	

* The components not listed here do not interfere.
** See text for explanation.

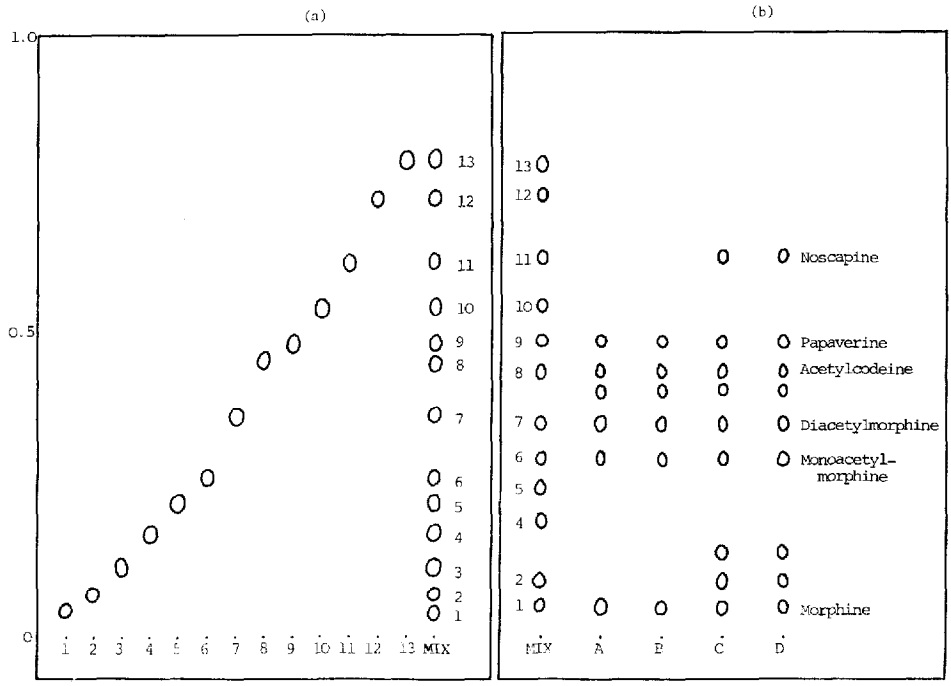

Fig. 1. Thin-layer chromatogram developed in chloroform–*n*-hexane–triethylamine (9:9:4): (a) individual standards and mixture of standards; and (b) seized heroin samples (A–D) and mixture of standards. 1 = Morphine; 2 = quinine; 3 = ephedrine; 4 = codeine; 5 = caffeine; 6 = 6-monoacetylmorphine; 7 = diacetylmorphine; 8 = acetylcodeine; 9 = papaverine; 10 = thebaine; 11 = noscapine; 12 = cocaine; 13 = methadone.

codeine); (ii) opium (morphine, codeine, noscapine, papaverine and thebaine); (iii) unadulterated heroin (the above five opiates, MAM, DAM and acetylcodeine); or (iv) adulterated heroin (the above eight opiates and possibly the five adulterants caffeine, cocaine, methadone, ephedrine and quinine).

Of the 35 systems listed in Table I, only 15 could be classified according to the categories listed, of which 14 were capable of resolving the opiates in urine (codeine and morphine) and only one of resolving the opium alkaloids. The remaining 20 systems could not be classified because of interference from the other components used in the evaluation. None of the reported systems were able to resolve the 13 test components. However, the information provided in Table I can be used for selecting a suitable solvent system if some of the opiates and adulterants are excluded from the analysis of an illicit heroin sample.

Fig. 1a shows the TLC of the eight opiates and five adulterants run individually and in a mixture using the new solvent system, chloroform–*n*-hexane–triethylamine (9:9:4). Table II shows the colours observed with the use of Marquis, acidified iodoplatinate and Dragendorff reagents. As the opiates and adulterants are visible under UV light (254 nm) and quinine is strongly fluorescent (light blue) at 366 nm, all 13 components were first located under UV light and subsequently identified on the basis of their R_F values and the colour produced with the spray reagents. The limit of detection of the individual components in the mixture was 0.1 μg.

TABLE II

COLOUR REACTIONS OF OPIATES AND ADULTERANTS WITH DIFFERENT REAGENTS

Compound	*Iodoplatinate reagent*	*Dragendorff's reagent*	*Marquis reagent*
Morphine	Blue	Orange	Violet
6-Monoacetylmorphine	Dark blue	Orange	Black
Diacetylmorphine	Dark blue	Orange	Black
Codeine	Blue	Red-orange	Dark blue
Acetylcodeine	Purple	Orange	Black
Noscapine	Purple	Orange	Green-black
Papaverine	Purple-brown	Orange	Maroon
Thebaine	Red-brown	Orange	Orange-red
Caffeine	Yellow	No colour	Light brown
Cocaine	Green-black	Pink	No colour
Methadone	Green-brown	Pink	Orange-red
Quinine	Black	Pink	No colour
Ephedrine	Brown	No colour	Yellow-brown

The applicability of this system was demonstrated by the analysis of two batches of illicit heroin samples. Fig. 1b shows the TLC of a mixture of the opiates and adulterants (excluding quinine) and the four overseas illicit samples (A–D) run under the same conditions. The individual components of the samples were identified by comparison with a standard mixture and are listed adjacent to sample D. The TLC system also resolved three other components that currently remain unidentified. None of the five adulterants was present in the illicit samples. The TLC analysis was confirmed by GC using an OV-210 column. Fig. 2a shows the chromatogram of the standard mixture of opiates with caffeine as the adulterant and Fig. 2b is the chromatogram of sample D. The peaks were identified from the retention times of the components of a standard mixture. All the opiates identified by TLC were also observed in GC, with the exception of morphine. Morphine and the three unidentified components were probably adsorbed by the column packing material and therefore eluted slowly.

A qualitative comparison of the chemical composition of the samples (A–D) in Fig. 1b suggests a classification into two groups: A + B and C + D, the latter group containing noscapine and other interfering substances. This qualitative assessment is supported by semi-quantitative GC data based on relative peaks areas. The relative peak-area percentages of the GC opiate peaks in each sample are shown in Table III. The data represent the mean of four analyses per sample and show the similarity in opiate composition between samples A and B or C and D.

The GC and TLC procedures mentioned above were also applied to a batch of 21 heroin samples that were seized locally. The TLC of a representative selection of the samples is shown in Fig. 3a, together with the standard mixture. The analysis indicated that all samples contained acetylcodeine and MAM but only some samples contained DAM. The absence of DAM from some samples could be due to its total breakdown during storage or shipment. None of the samples contained any other unidentifiable components. The TLC analysis was confirmed by GC. Fig. 3b illus-

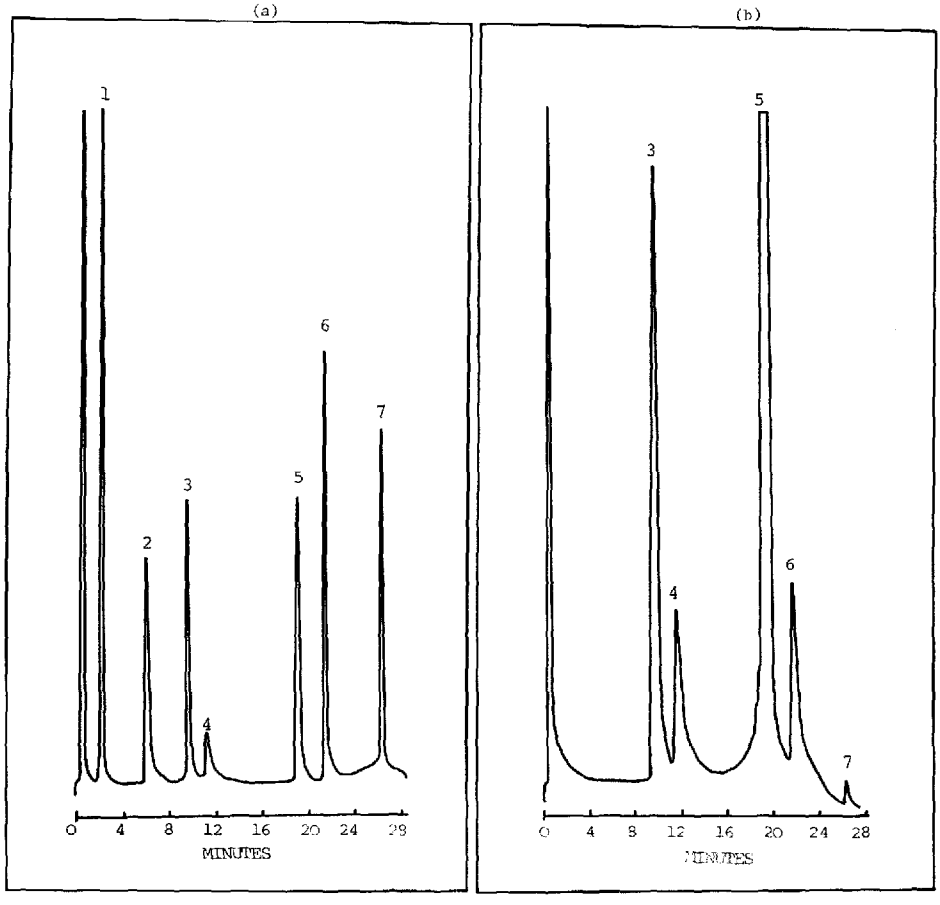

Fig. 2. Gas chromatogram using an OV-210 column: (a) mixture of opiates and caffeine; and (b) seized heroin sample D. See text for conditions. 1 = Caffeine; 2 = codeine; 3 = acetylcodeine; 4 = 6-monoacetylmorphine; 5 = diacetylmorphine; 6 = papaverine; 7 = noscapine.

trates this by showing the gas chromatogram of one of the samples (No. 11). The peaks were identified by comparison with the chromatogram of a standard mixture (Fig. 2a).

Semi-quantitative analysis of the 21 samples based on peak-area percentages

TABLE III

RELATIVE OPIATE COMPOSITION OF SEIZED HEROIN (A–D) BASED ON GC PEAK AREAS

Sample	*Relative percentage*				
	Acetylcodeine	*MAM*	*Heroin*	*Papaverine*	*Noscapine*
A	7.9	2.1	89.3	0.3	–
B	7.7	3.2	88.2	1.0	–
C	7.5	4.2	85.7	1.6	1.8
D	7.3	4.6	84.8	1.5	1.7

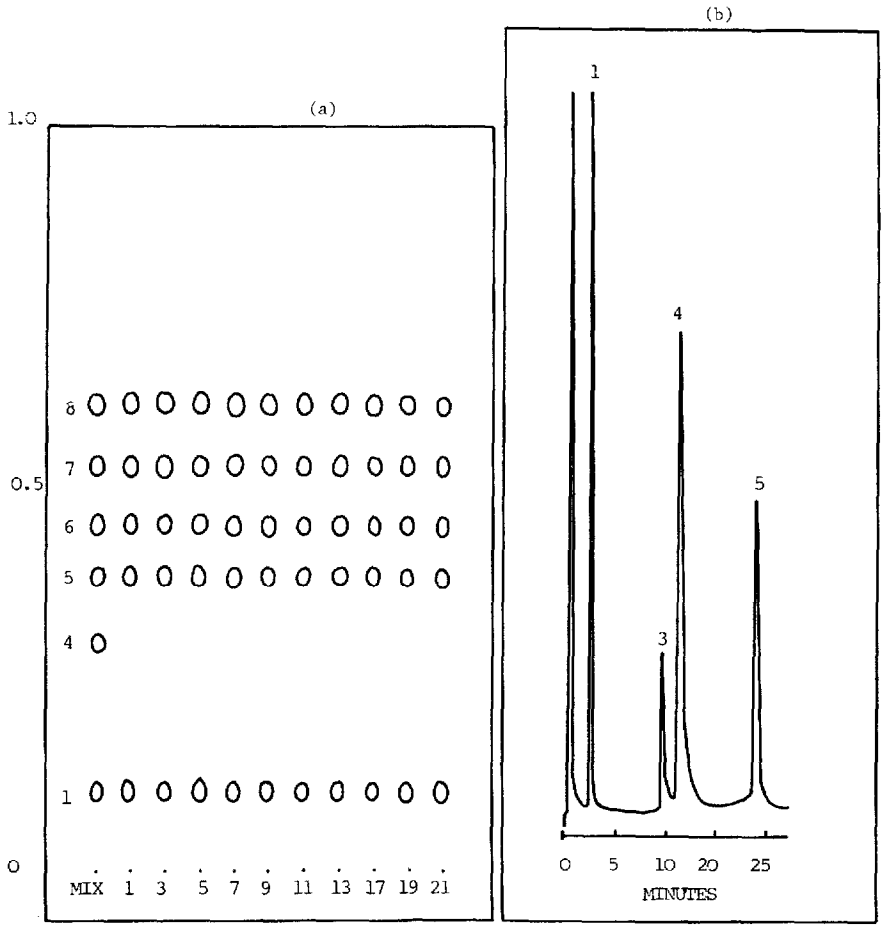

Fig. 3. (a) Thin-layer chromatogram of some locally seized heroin samples. See Fig. 1 for identification. (b) Gas chromatogram of one of the locally seized heroin samples (No. 11). See text for conditions and Fig. 2 for identification.

TABLE IV

GROUPING OF ILLICIT HEROIN SAMPLES BASED ON GC ANALYSIS

Values given are relative peak-area percentages.

Set	*Group*	*No. of samples*	*Caffeine*	*Acetylcodeine*	*MAM*	*DAM*	*MAM + DAM*
A	1	6	23.9 ± 1.5	7.9 ± 1.7	1	68.8 ± 2.7	–
	2	3	21.3 ± 2.3	15.0 ± 1.5	1	63.7 ± 0.9	–
	3	2	59.1 ± 2.1	6.6 ± 0.7	34.4 ± 1.5	ND*	–
B	4	2	32.3 ± 0.8	8.9 ± 0.1	–	–	58.8 ± 0.7
	5	2	37.3 ± 1.0	8.5 ± 1.5	–	–	54.3 ± 0.6
	6	2	42.9 ± 0.2	8.0 ± 2.0	–	–	49.1 ± 1.2
C	7	2	–	11.4 ± 0.9	1	88.6 ± 0.9	–

* ND = Not detected.

permitted the classification of 19 samples into seven groups. Two samples could not be classified because of their widely differing compositions. The seven groups shown in Table IV are divided into three sets according to the nature of the comparison. In set A the samples were grouped on the basis of their relative (and individual) opiate and caffeine compositions. The samples in set B could not be classified in a similar manner because of the varying amounts of MAM and DAM. However, if it is assumed that MAM originated from the breakdown of DAM, then the samples can be grouped according to their combined DAM and MAM composition. In set C the two samples have similar opiate compositions without any adulterant.

A comparison of the overseas and local samples (Figs. 1b and 3a) shows the former to contain morphine, DAM, MAM, acetylcodeine, noscapine, (in some) papaverine and other unidentified components. In contrast, however, the local samples contained fewer opiates and only caffeine as the adulterant. The absence of noscapine and papaverine from the latter suggests that the extraction procedures utilized in the illicit preparation were more effective. Caffeine was added subsequently, probably at the source of manufacture.

ACKNOWLEDGEMENTS

This study was jointly funded by the United Nations Fund for Drug Abuse Control (UNFDAC) and the Government of Malaysia.

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