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TECHNICAL NOTE

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Analysis of a Simulated Heroin Distribution Chain by HPLC

ABSTRACT: A heroin distribution chain was simulated by taking three different seizures and preparing four additional samples from each seizure by adding a paracetamol-caffeine mixture in varying amounts, resulting in three different batches each composed of five samples. All of the samples from the three batches were analyzed using HPLC with a UV-PDA detector at a wavelength of 230 nm. The area ratio of various opium alkaloids, acetylation products and components were compared. From the results of the UV area ratios, the fifteen samples could readily be separated into three batches of five samples, with each batch of five samples having a common origin.

KEYWORDS: forensic science, heroin, heroin comparison, common origin, high performance liquid chromatography

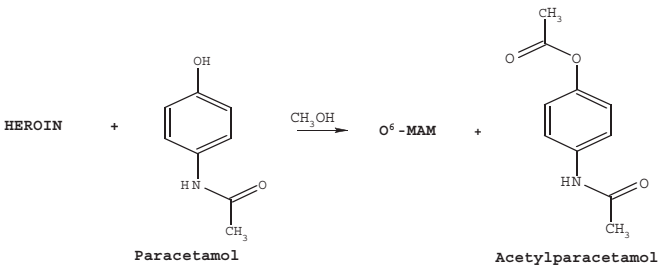
Israel is a drug consuming country. Different drugs are smuggled into the country by land, air and sea. Heroin is the second most common drug, constituting approximately 25% of all seizures (cannabis and hashish combined constitute approximately 65% of all seizures).

Heroin is synthesized from morphine, which in turn is isolated from opium. However, opium is a mixture of more than 35 alkaloids, the most important being morphine, codeine, narcotine, papaverine and thebaine. Their concentrations vary depending upon the soil, climate, growing conditions and timing when the latex from the opium poppy is harvested (1). The synthesis of heroin from morphine proceeds via the intermediate O^3 – monoacetylmorphine (O^3–MAM) and heroin decomposes to morphine via the intermediate O^6 – monoacetylmorphine (O^6–MAM) (2–6).

The separation of morphine from the various opium alkaloids in clandestine laboratories is inefficient and therefore contains numerous impurities. During the heroin synthesis process some of these impurities undergo acetylation (7), such as codeine to acetylcodeine, or decomposition, such as thebaine to dimethoxyacetoxyphenanthrene.

Variations in the isolation of morphine and variations in its acetylation create additional differences, which are expressed in the final composition of the drug batch (2,8). Variations in the composition of street heroin have been reported in numerous articles (9–14). In a survey on thousands of illicit heroin seizures in Israel in 1992, the two most common adulterants found were caffeine and paracetamol. Over 80% of the "user" seizures (0.1–1.0 g) contained caffeine and/or paracetamol (15). This trend has continued and in 2003 caffeine and/or paracetamol were found in over 90% of the illicit heroin seizures. It follows that in a given heroin batch one would expect to find similar ratios between the opium alkaloids and reaction products even if an inert additive or diluents were added to increase the volume (16–18).

However, unusual diluents or adulterants and improper storage conditions could affect the hydrolytic rate of decomposition of heroin to O^6–MAM and this should be taken into account appropriately. Care must also be taken that the selected method of analysis does not create artifacts in the results. For example, if paracetamol was used as an additive and the batch was analyzed by GC or GC/MS using methanol as the solvent, acetylation of the paracetamol could occur (19) according to the following reaction:



The possibility of dry acetylation of codeine to acetylcodeine by paracetamol should also not be overlooked.

Numerous chromatographic or combined chromatographic methods, such as GC or HPLC (9,12,13,20,21), are capable of resolving a mixture or simulated mixture (22) into its individual components. Each method has its own advantages and disadvantages. Gas chromatography has higher resolution than HPLC but is prone to thermal decomposition and transacetylation. On the other hand HPLC is not prone to these limitations but when a UV detector is used the possibility of artifacts due to free radical reactions or decomposition exists (23). Other separation methods, such as those based on

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capillary electrophoresis (CE) have also been used for the analysis of illicit heroin seizures (24,25). In this work HPLC with a UV-photo diode array (PDA) detector was chosen as the most appropriate method of analysis. Simulation of the distribution chain was performed on three original seizures, simulating the importer, by the addition of varying amounts of a paracetamol-caffeine mixture, simulating intermediate and street dosages.

Methods

Standards and Chemicals

For the simulated heroin distribution chain we used three different street heroin powders from three different seizures, cases number: 12926-2000 (batch 1), 15965–2000 (batch 2), 18500–1997 (batch 3) and a mixture of paracetamol-caffeine from a police seizure, case number 16863–2000. For the correction factor we used the heroin standard (diacetylmorphine HCl), Alltech Associates inc. and O^6–MAM, synthesized from morphine and checked for purity by GC/MS. Solvents for HPLC were HPLC grade, Merck, Israel.

Apparatus

The HPLC data were obtained using a Waters model 600S instrument with a UV detector type PDA model 996 pump. Automatic sampling was performed using a model 717 plus autosampler. Samples were prepared by weighing approximately 16 mg of the powder, dissolving it in 4.0 mL of methanol and placing it in an ultrasonic vibrator for fifteen minutes. The solution was diluted 1/10 with water, filtered using a 0.45 micron filter and 10 μL injected into the HPLC (corresponding to approximately 4 μg of sample). HPLC analysis conditions were as follows: flow rate 1.8 mL/min, mobile phase—a mixed gradient of HPLC grade water, acetonitrile and methanol, each containing 2M sulfuric acid in a ratio of 1/1000 (see Table 1 for HPLC gradient variation), column – Merck Li Chrospher 60 – RP- select B. LiCho CART – 125 – 4 – EcoPach (5 μm), detector wave length 230 nm.

Procedures

The simulated heroin distribution procedure was performed on three actual heroin seizures. The composition of the three original seizures is given in Table 2. Seizure 1 was known to originate from Lebanon and contained caffeine and paracetamol as additives. Seizure 2 was of an unknown origin and did not contain any additives. Seizure 3 was also known to originate from Lebanon and contained caffeine as an additive. To each of the above three seizures varying amounts of a mixture of paracetamol and caffeine (having an HPLC peak area ratio at 230 nm of 4.5:1 paracetamol:caffeine) were added creating four additional samples of varying concentrations. The approximate ratios prepared of the original seizure to the paracetamol-caffeine mixture are summarized in Table 3. The five samples from each batch (Labeled A, B, C, D, E) were analyzed by HPLC and the area ratios of various components calculated using the program Microsoft EXCEL 2000 (Table 4).

TABLE 1—*HPLC gradient composition versus time.*

	Mobile Phase %		
Time (min)	Water	Acetonitrile	Methanol
0	96	0	4
4	96	0	4
19	85	13	2
25	85	13	2
25.5	85	11	4
33	85	11	4
35	96	0	4
42	96	0	4

TABLE 2—*Percent composition of the three original seizures.*

Seizure	H	O^6-MAM	O^3-MAM	AC	N	PAPA	CAFF	PARA
1A	40.1	1.8	~0.4	4.8	19.7	2.9	14.8	0.2
2A	67.9	4.8	~0.8	7.4	13.9	3.1	N.D.	N.D.
3A	39.8	4.0	~1.0	5.6	27.0	3.2	22.4	N.D.

N.D. = Not detected.
~ = Approximate value.

TABLE 3—*Ratio of original seizure to paracetamol-caffeine additive in simulated samples.*

Sample	Original Seizure	Paracetamol/Caffeine Mixture
A	4	0
B	3.5	0.5
C	3	1
D	2	2
E	1	3

Results and Discussion

Heroin is a relatively stable compound when stored under suitable conditions. A high concentration of O^6-MAM indicates decomposition of the heroin, while the presence of O^3-MAM indicates incomplete acetylation of the morphine. To prevent distortions of component ratios as a result of heroin decomposition to O^6-MAM due to hydrolysis or reactions with other components in the mixture, the total morphine content was calculated, defined as $H + O^3\text{-MAM} + O^6\text{-MAM} + M$, and divided by the HPLC peak area of a more stable alkaloid namely narcotine. In addition a correction factor was determined to compensate for the HPLC detector sensitivity difference between O^6-MAM and heroin. The peak area ratio of H/O^6-MAM on a weight : weight basis was found to be 0.917. Therefore all O^6-MAM area results in the total morphine term were calculated as heroin according to the following equation:

$$H = O^6\text{-MAM}\,(0.917)$$

A similar correction factor for O^3-MAM was considered unnecessary since the HPLC peak area of O^3-MAM was always less than 1.4 % of the peak area of heroin. Morphine, codeine and thebaine were not detected in the three original seizures. Typical HPLC chromatograms are illustrated in Fig. 1.

The ratio of heroin to total morphine was calculated as a measure of the amount of heroin present as a function of the "potentially available heroin". In a given heroin seizure, heroin, O^3-MAM, O^6-MAM and morphine may be present in addition to other compounds. Under ideal synthesis and storage conditions, of these four compounds only heroin would be present. If the synthesis had been complete, all the morphine and O^3-MAM would have been converted to heroin. If no hydrolysis had occurred, O^6-MAM and morphine would not be present. Thus these three compounds all had the "potential" to be converted to heroin.

The following additional ratios were also calculated, based on HPLC peak areas: heroin/narcotine (H/N), acetylcodeine/heroin

TABLE 4—*HPLC area ratios at a wavelength of 230 nm.*

Column Sample	1 H/N	2 AC/H	3 (H + AC + O^6-MAM + O^3-MAM + M)/N	4 PARA/CAFF	5 PAPA/N	6 (O^6-MAM + O^3-MAM + H + M)/N [Total Morphine/N]	7 AC/N	8 H(O^6-MAM + O^3-MAM + H)
1A	0.934	0.101	1.075	0.022	0.094	0.981	0.094	95.2%
1B	0.822	0.110	0.999	0.887	0.120	0.908	0.091	91.4%
1C	0.806	0.105	0.976	1.579	0.104	0.891	0.085	90.5%
1D	0.807	0.101	0.935	3.263	0.099	0.853	0.081	94.5%
1E	0.999	0.099	1.126	3.993	ND[1]	1.027	0.098	97.2%
$\bar{x} \pm \sigma$	0.874 ± 0.088	0.103 ± 0.005	1.022 ± 0.077	1.949± 1.649	0.104± 0.011	0.932 ± 0.070	0.090± 0.007	93.8% ± 2.8%
2A	2.241	0.092	2.619	ND[2]	0.141	2.414	0.206	92.8%
2B	2.215	0.091	2.581	4.531	0.135	2.380	0.201	93.0%
2C	2.164	0.087	2.529	4.523	0.151	2.341	0.189	92.4%
2D	2.200	0.085	2.516	4.516	0.130	2.330	0.186	94.4%
2E	2.444	0.077	2.751	4.540	0.156	2.564	0.187	95.3%
$\bar{x} \pm \sigma$	2.253± 0.110	0.086± 0.006	2.599 ± 0.094	4.528± 0.010	0.143± 0.011	2.406 ± 0.094	0.194± 0.009	93.6% ± 1.2%
3A	0.589	0.118	0.726	ND[3]	0.071	0.656	0.070	89.7%
3B	0.594	0.112	0.688	0.595	0.059	0.621	0.066	95.6%
3C	0.602	0.104	0.679	1.262	0.048	0.616	0.063	97.8%
3D	0.561	0.116	0.657	2.700	0.066	0.592	0.065	94.8%
3E	0.585	0.106	0.647	3.794	0.079	0.584	0.062	100.0%
$\bar{x} \pm \sigma$	0.586± 0.015	0.111± 0.006	0.679 ± 0.031	2.088± 1.437	0.065± 0.012	0.614 ± 0.028	0.065± 0.003	95.6% ± 3.9%
Total $\bar{x} \pm \sigma$	1.238± 0.757	0.100± 0.012	1.434 ± 0.868	2.785± 1.700	0.104± 0.036	1.317 ± 0.811	0.116± 0.058	94.3% ± 2.8%

$\bar{x} \pm \sigma$ = average value ± standard deviation.
ND[1] = papaverine not detected, not included in average value and standard deviation.
ND[2] = paracetamol and caffeine not detected, not included in average value and standard deviation.
ND[3] = paracetamol not detected, not included in average value and standard deviation.

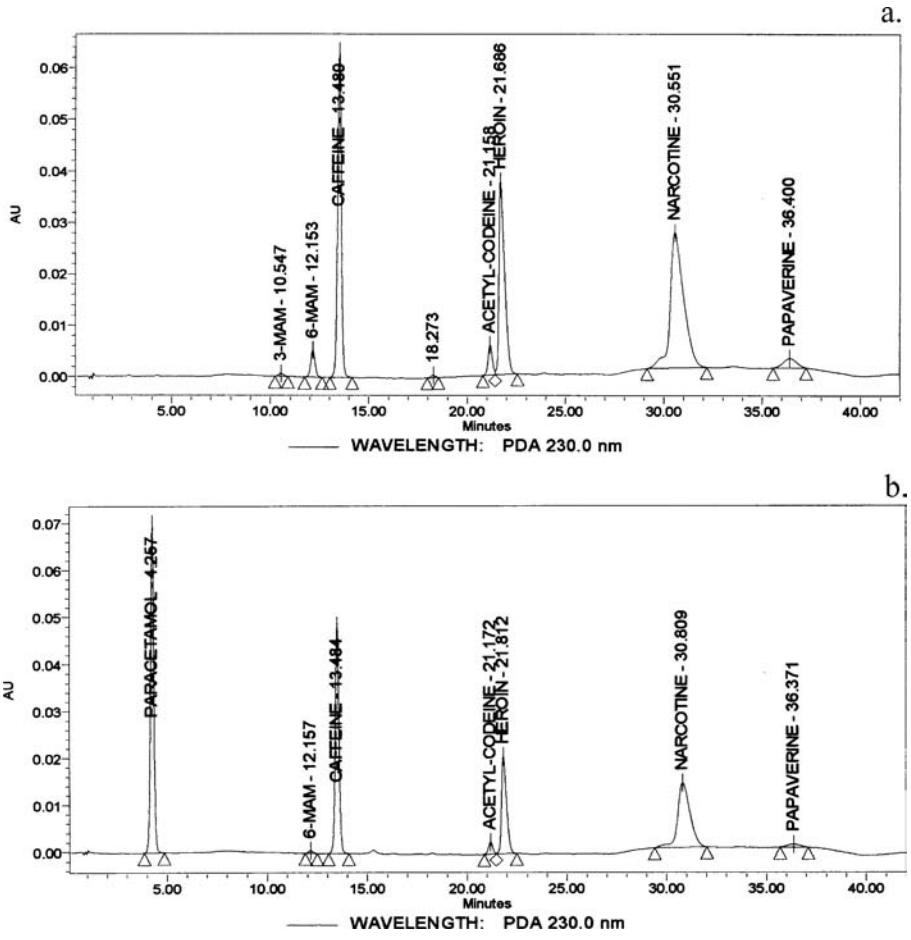

FIG. 1—*HPLC chromatogram of: (a) original seizure 3 (b) original seizure 3 after adding the paracetamol-caffeine mixture.*

(AC/H), (H + AC + O^3-MAM + O^6-MAM + M)/N, paracetamol/caffeine (PARA/CAFF), papaverine/narcotine (PAPA/N) and AC/N (see Table 4). Calculating component ratios, as opposed to the concentration of components, has the advantage of obviating the need to prepare calibration graphs.

In Table 4, it is clear from the data in columns 1, 3, 5, 6 and 7 that there are three distinct batches and that the addition of the paracetamol-caffeine mixture in varying ratios did not obscure these distinctions. The data in column 8, measuring the ratio of the heroin content as a function of the potentially available heroin, could not be used as a basis for distinguishing between the three batches, because in all three batches the average value was greater than 90%, with strongly overlapping standard deviations. In any given batch, there was no consistent decrease in this ratio as the amount of additives increased, indicating that no significant amount of heroin decomposition occurred as a result of the additives. In column 2 (AC/H), it is possible to distinguish batch 2 from batches 1 and 3, but the differences between batches 1 and 3 are less clear. In column 4 (PARA/CAFF), in seizure 2 (which did not contain paracetamol or caffeine in the original seizure), the various samples containing the additives all produced the same results, namely the original ratio of the paracetamol:caffeine. Thus, the diluted samples in batch 2 are readily distinguished from batches 1 and 3. In contrast to this, in batches 1 and 3, which contained caffeine and/or paracetamol in the original seizure, the ratio varied in each of the samples containing the additive, as would be expected, and batches 1 and 3 could not be distinguished from each other, based on this ratio.

Upon examining the standard deviation of the area ratios within each batch, in column 6 (total morphine/N) for example, the standard deviations are low, having values of 0.070, 0.094 and 0.028 compared to a high standard deviation for the total population (all of the samples in the three batches) of 0.811. In contrast to this, in column 2 (AC/H), the standard deviations within the batches are 0.005, 0.006 and 0.006 compared to a total standard deviation of 0.012. When the standard deviation of the total population is large compared to the standard deviation of the batches, the results are significant for comparison purposes, whereas when the standard deviation of the total population is low compared to the standard deviation of the batches, the results are less significant for comparison purposes.

Conclusions

Three batches of heroin, each consisting of an original seizure and four diluted samples, were readily distinguished from each other by HPLC by comparing peak area ratios of various alkaloids, acetylation products and components. The additive used to simulate a distribution chain, consisting of a paracetamol – caffeine mixture, did not create any significant change in the ratios of the various alkaloids and acetylation products in the three original seizures. Thus it was possible to correlate the diluted samples to an original seizure. Although the database is small, there are no indications that any reactions occurred between the additive and the components in the original seizures.

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