

Detection of Acetylcodeine in Urine as an Indicator of Illicit Heroin Use: Method Validation and Results of a Pilot Study

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Background: Acetylcodeine (AC), an impurity of illicit heroin synthesis, has been suggested as an interesting biomarker of illicit heroin use.

Methods: Procedures were developed for quantification of (a) morphine, 6-monoacetylmorphine (6-AM), and codeine in urine and (b) diacetylmorphine and AC in urine. Solid-phase extraction of the analytes was performed, and the extracted analytes were analyzed by selected-ion monitoring with gas chromatography–mass spectrometry. This procedure required prior derivatization with propionic anhydride.

Results: Different validation parameters were determined, such as linearity, reproducibility, extraction recoveries, and cutoffs. Seventy-one urine specimens of illicit heroin abusers and 44 urine specimens of subjects in a heroin maintenance program were analyzed. AC was detected in 85.9% of the samples of the first group but not in any of the samples from subjects taking medical heroin. In the two groups, there were 94.4% and 84.1% 6-AM positive urine specimens, respectively. Detection times were determined for AC and codeine by parallel administration of heroin containing various percentages of AC to four voluntary patients in a heroin maintenance program. The measured detection times were 8 and 23 h for AC and codeine, respectively.

Conclusions: These results indicate that, together with detection of 6-AM in urine, AC is a suitable marker of illicit heroin use.

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Several reports have described the analysis of opiates in urine (1–3) to confirm the illicit consumption of heroin

and to distinguish heroin use from codeine or morphine use. However, interpretation of positive morphine results can be difficult because of the presence of opiate alkaloids in medicines and foods. For example, morphine and codeine are present in many preparations for the treatment of pain and cough suppression. In addition, they are found in various amounts in opium poppy seed (4), a common ingredient of bakery products. Ingestion of these products leads to excretion of codeine and morphine in urine.

As illustrated in Fig. 1, both codeine and heroin are metabolized into morphine, which is then excreted in the urine. Therefore, detection of morphine in urine can result from intake of heroin, morphine, codeine, or poppy seeds.

6-Monoacetylmorphine (6-AM)⁴ in urine has been suggested as a specific marker of heroin abuse, and several methods for its detection have been reported (5–8). The detection time measured for 6-AM is short (<8 h), whereas the detection times for other metabolites, such as morphine, are longer.

In addition to 6-AM, acetylcodeine (AC) has been suggested recently as another marker of illicit heroin use (9). AC is a manufacturing impurity (1–15%) of heroin (10) and is metabolized into codeine and, subsequently, into morphine (Fig. 1).

In 1994, the Swiss Federal Office of Public Health started a new heroin maintenance program in which addicted patients receive heroin under governmental supervision. Here, AC could be useful in monitoring addicts enrolled in such programs. Because the maintenance heroin administered is pure (<0.1% AC), the presence of AC in the urine of these patients indicates that they may be supplementing their supervised heroin doses with illicit heroin.

The role of AC in urine as a specific indicator of illicit

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⁴ Nonstandard abbreviations: 6-AM, 6-monoacetylmorphine; AC, acetylcodeine; LSD, D-lysergic acid diethylamide; GC-MS, gas chromatography–mass spectrometry; and LOQ, limit of quantification.

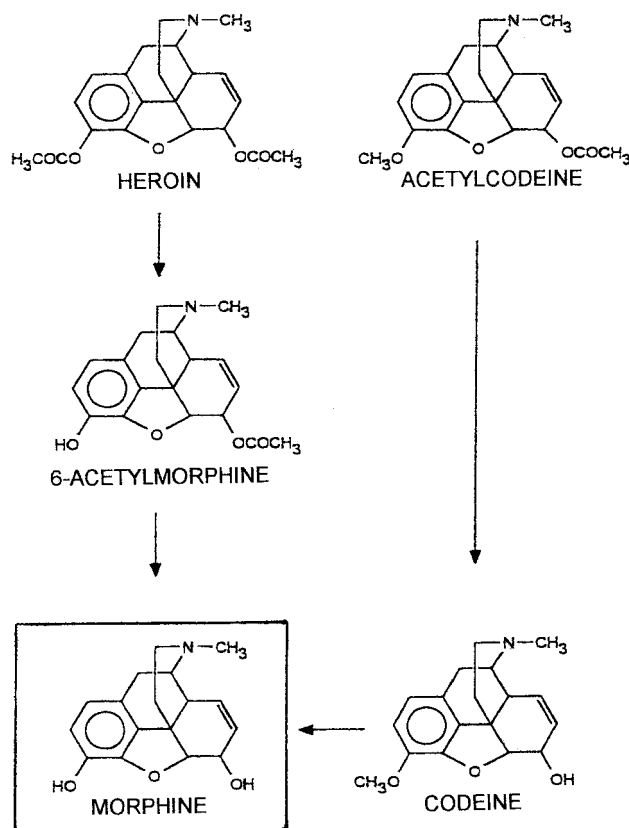


Fig. 1. Metabolic pathways for heroin and AC.

heroin use depends on two main factors: (a) the availability of a reliable and sensitive analytical method for AC detection and, subsequently, availability of other opiate detection methods; and (b) an adequate detection time for AC and codeine in urine. Two separate procedures were validated and applied to urine specimens from patients in a heroin maintenance program and from illicit heroin users.

This report also describes a study on AC excretion in urine. Four volunteer patients in a heroin maintenance program agreed to take heroin with various percentages of AC, which allowed us to determine detection times for AC and codeine.

Materials and Methods

CHEMICAL REAGENTS AND INSTRUMENTATION

The acid, buffer, solvents, and pyridine were provided by Merck. Propionic anhydride was supplied by Aldrich.

Table 1. Group characteristics.

Group	Male	Female	Age, ^a years	Heroin, ^a g/day
Medical heroin consumers (n = 44)	32	12	33 (21–52)	0.46 (0.12–0.75)
Illegal heroin consumers (n = 74)	54	20	32 (18–50)	1.2 (0.1–4.0)

^a Mean (range).

Table 2. Parallel consumption (%) of the two groups.

	Illegal heroin consumers (n = 74)	Medical heroin consumers (n = 44)
Cocaine	89.2	15.9
Cannabis	41.9	59.1
Methadone	41.9	34.1
Benzodiazepines	29.7	22.7
Methaqualone	9.5	9.1
Barbiturates	5.4	2.3
Tricyclic antidepressants	4.1	6.8
LSD	4.1	0
Amphetamines	1.4	0
No parallel consumption	1.4	18.2

Drugs and nalorphine were obtained from Cambridge Isotope Laboratories and Sigma. Bond Elut Certify cartridges (sorbent type, C₈ and SO₃H; weight, 130 mg) were obtained from Varian and used for the extractions. Urine analyses were performed using a Hewlett Packard 5890 gas chromatograph equipped with a mass-selective detector (Model 5972; Hewlett Packard) that operated in electron-impact mode with an energy of 70 eV.

DOSE AND SPECIMEN COLLECTION

Urine specimens were obtained from illegal heroin users and subjects enrolled in the Prove Program, a medical heroin treatment program set up by the Swiss Federal Office of Public Health. Volunteers of the Prove Program received 120–750 mg of heroin intravenously per day. Urine specimens were collected at least 3 months after the beginning of the treatment. For the group of illegal heroin consumers, the indicated heroin daily dose was based on the consumers' declarations.

Immunoassay screening was performed on all urine specimens using the following tests, cutoff values, and reference substances: amphetamines (*d*-methamphetamine, 1000 µg/L); barbiturates (secobarbital, 200 µg/L); benzodiazepines (nordazepam, 100 µg/L); cannabis (11-nor-Δ⁹-THCCOOH, 50 µg/L); cocaine (benzoylecgonine, 300 µg/L); methadone (300 µg/L); methaqualone (300 µg/L); tricyclic antidepressants (nortriptyline, 300 µg/L); and *D*-lysergic acid diethylamide (LSD, 0.5 µg/L).

Three heroin consumers were excluded from the study because only morphine was found in their urine and the

Table 3. Dose characteristics for the four patients receiving medical heroin.

Subject	Dose, mg			
	Morning	Noon	Evening	Per day
A	210	210	230	650
B	200	200	200	600
C	250	250	270	770
D	150	110	150	410

Table 4. Linearity and LOQ in urine analysis.

	Linearity, <i>R</i>	Range, $\mu\text{g/L}$	Assigned LOQ, $\mu\text{g/L}$	Amount found		Internal ions	Ion ratio (<i>n</i> = 8)	
				Mean, $\mu\text{g/L}$	CV, %		Mean	CV, %
Morphine	0.9985	10–30 000	10	9.7	7.0	397/341	0.409	2.7
6-AM	0.9908	10–30 000	10	9.1	9.1	383/327	0.487	4.9
Codeine	0.9975	10–30 000	10	11.9	6.6	355/282	0.962	2.2
AC	0.9937	1–500	1	0.8	8.4	341/282	1.218	5.5
Heroin	0.9921	5–500	5	4.0	7.1	369/327	0.680	2.8

concentration was $<100 \mu\text{g/L}$. This lower concentration could be explained by too long of a period between heroin consumption and urine sampling.

Subject characteristics and parallel consumption (results of immunoassay screening) for the two groups are summarized in Tables 1 and 2. A third group included four voluntary patients in a heroin maintenance program who agreed to take heroin containing various percentages of AC. To simulate parallel consumption of illicit heroin, they received heroin containing various percentages of AC during 36 days in the following manner: (a) between days 2 and 9, heroin contained 9% AC; (b) during days 12 and 13, heroin contained 5% AC; and (c) between days 19 and 26, heroin contained 3% AC. Pure heroin was administered on days 1, 10, 11, 14–18, and 27–36.

All patients received their daily heroin in three doses, and only the morning dose contained AC. Table 3 summarizes dose characteristics for the four patients. During days 2, 12, and 19, urine specimens were collected at 0, 1, 3, 6, 12, and 24 h after the morning dose. Urine collections performed at 0, 6, 12, and 24 h were just prior to administration of the next dose of heroin. All urine specimens were stored at -20°C until analysis. We found that AC was very stable in these storage conditions because there was no loss after 6 months of storage.

The study was conducted according to the guidelines for the protection of human subjects, and each volunteer provided informed consent.

SAMPLE PREPARATION

Nalorphine (300 μL of a 25 mg/L solution) was added to 1 mL of urine for procedure A (morphine, 6-AM, and codeine), and nalorphine (200 μL of a 2.5 mg/L solution) was added to 2 mL of urine for procedure B (heroin and AC).

Urine specimens were then extracted by the same technique with Bond Elut Certify columns. This consisted of conditioning the column with 2 mL of methanol, followed by 2 mL of deionized water. Samples were added to the columns, and the columns were then rinsed with 2 mL of deionized water, 2 mL of 0.1 mol/L acetate buffer (pH 4), and 2 mL of methanol. Columns were dried under reduced pressure (10 mmHg) for 5 min, then eluted with 2 mL of methylene chloride-isopropanol (4:1 by

volume) containing 20 mL/L ammonium hydroxide. The eluate was dried under nitrogen.

Pyridine and propionic anhydride (100 μL each) were then added, and samples were heated at 60°C for 30 min. After derivatization, the reagent was dried under nitrogen, and the samples were reconstituted with 50 μL of ethyl acetate.

GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC-MS)

For GC, a DB-5MS capillary column (15 m \times 0.25 mm; 0.25- μm film thickness; J & W Scientific) was used with helium as the carrier gas. The following temperatures were applied: 170°C maintained for 1 min; ramped to 240°C at $20^\circ\text{C}/\text{min}$, to 256°C at $2^\circ\text{C}/\text{min}$, and to 270°C at $10^\circ\text{C}/\text{min}$; and then held at 270°C for 0.6 min. The injector temperature was 270°C , and injection was made in splitless mode. The interface temperature was 280°C .

The sample (2 μL) was injected into the GC-MS system, which was operating in selected-ion monitoring mode. The electron multiplier voltage was set at the EI-tune voltage for procedure A and at +200 V above EI-tune voltage for procedure B.

The following ions were monitored: for procedure A, morphine (*m/z* 397 and 341), 6-AM (*m/z* 383 and 327), codeine (*m/z*, 355 and 282), and nalorphine (*m/z* 423 and 367); and for procedure B, heroin (*m/z* 369 and 327), AC (*m/z* 341 and 282), and nalorphine (*m/z* 423 and 367).

The internal ratio for each compound was monitored. Quantification was based on the peak-area ratios (the first

Table 5. Recovery and reproducibility in urine analysis (n = 6).

	Concentration, $\mu\text{g/L}$	Recovery, %	CV, %
Morphine	500	101.1	2.1
	2500	107.3	5.4
6-AM	500	83.6	3.6
	2500	85.4	2.2
Codeine	500	73.4	4.1
	2500	94.5	3.9
AC	25	82.5	7.4
	250	81.0	2.5
Heroin	25	88.1	4.7
	250	84.5	3.0

Table 6. Opiate concentrations ($\mu\text{g/L}$) in urine of illicit heroin users.

Number	Morphine	6-AM	Codeine	AC	Heroin	Number	Morphine	6-AM	Codeine	AC	Heroin
1	3612	11	237	0	0	39	3036	2463	0	0	470
2	1392	356	85	10	6	40	12 707	3197	2292	166	63
3	2646	584	138	18	4	41	847	68	32	7	0
4	5306	1071	362	22	0	42	15 440	6213	3871	215	42
5	10 376	839	3282	12	0	43	18 830	3426	2101	124	51
6	9221	404	2829	13	0	44	1795	889	228	18	8
7	393	0	47	0	0	45	2672	2261	405	314	305
8	1064	347	181	5	0	46	1312	1046	121	25	10
9	3272	1393	702	106	138	47	3686	803	637	82	13
10	3605	2005	642	201	14	48	3334	1099	4260	215	0
11	1095	159	183	3	0	49	6592	1179	554	45	44
12	25 240	6610	4000	505	206	50	12 962	6295	2112	359	148
13	2858	4465	410	125	99	51	276	10	43	0	0
14	295	0	42	0	0	52	4316	257	1483	15	0
15	16 906	5254	3029	117	75	53	13 176	3650	1959	259	57
16	10 266	7711	2097	2710	10 265	54	4872	1929	329	40	17
17	2354	777	610	21	13	55	18 076	13 783	3166	1329	555
18	39 740	16 630	9412	2264	598	56	1674	868	223	35	14
19	265	24	75	3	0	57	9095	1591	1313	68	27
20	541	18	345	0	0	58	1068	563	103	14	0
21	11 003	1234	1115	28	0	59	35 180	11 330	6080	258	0
22	3283	678	657	15	16	60	14 793	4188	3176	240	80
23	15 822	3213	2699	118	50	61	3336	327	396	32	15
24	3363	925	581	59	14	62	8276	2028	2021	111	83
25	3958	577	893	47	13	63	7044	2174	897	31	17
26	13 656	5485	2348	194	137	64	10 458	7354	1929	265	79
27	1336	0	54	0	0	65	3705	1679	717	66	20
28	11 324	3260	2322	211	75	66	10 478	626	1493	13	0
29	25 490	16 900	3440	1062	119	67	16 860	3283	264	6	0
30	14 241	2932	2044	374	138	68	9277	1457	1078	33	0
31	411	0	0	0	0	69	184	56	12	0	0
32	4777	2420	8174	1387	385	70	4984	182	433	5	0
33	694	11	224	0	0	71	1017	619	82	11	0
34	7364	829	179	25	0	Mean	8571	2549	1541	204	205
35	15 822	5916	2485	166	82	Median	4872	1071	702	35	13
36	29 740	454	3830	110	0	Maximum	39 740	16 900	9412	2710	10 265
37	23 030	259	1021	30	0	Minimum	184	0	0	0	0
38	21 430	340	4860	99	0	Rate, %	100	94.4	97.2	85.9	57.8

ion listed for each compound was used) of the analytes to the internal standard (nalorphine).

VALIDATION PROTOCOL

Extraction recovery was determined by adding the analytes to drug-free urine at low and high concentrations ($n = 6$). After extraction, nalorphine was added as an external standard, and peak-area ratios were then compared with unextracted calibrators of equal concentrations in methanol or in acetonitrile for AC.

Reproducibility (within-run precision) was determined by analysis of a low and high concentration of each analyte on the same day ($n = 6$). Seven-point calibration curves for each analyte were analyzed to determine method linearity. The limit of quantification (LOQ) was determined as the lowest concentration yielding a result within $\pm 20\%$ of the target concentration and with a CV $<10\%$.

Results

These two procedures permitted the determination of five opiates: morphine, 6-AM, codeine, heroin, and AC. The analytes were determined with the following retention times: for procedure A, 8.60 min (morphine), 7.65 min (6-AM), and 6.55 min (codeine); and for procedure B, 6.75 min (heroin) and 5.85 min (AC).

Satisfactory validation data were achieved for linearity, recovery, and reproducibility. Linearity, accuracy, and precision for the two procedures were measured at the LOQ values, which are listed in Table 4. Assigned LOQ values were therefore used as cutoffs in the rest of the study with the additional condition that the internal ion ratio should correspond to $\pm 20\%$ of the value (given in Table 4) for reference standards. Extraction recovery and precision are given in Table 5 for two concentrations. CVs were generally $\leq 7.4\%$.

Seventy-one urine specimens from illicit heroin users and 44 urine specimens from medical heroin users were analyzed by the two procedures described above. Quantitative results, as well as the respective median, mean, and extreme values for all opiates, are listed in Tables 6 and 7. In these two groups, respectively, 94.4% and 84.1% 6-AM-positive urine specimens were found, again demonstrating that 6-AM is an good biomarker of heroin use. AC was detected in 85.9% of the samples from illicit heroin users but not in any of the samples from patients undergoing heroin maintenance. These results indicate that AC is a good biomarker of illicit heroin use, as demonstrated previously by O'Neal and Poklis (10). These authors found a positive relationship between AC and 6-AM concentrations in urine ($r = 0.878$). In our case, a positive relationship between AC and 6-AM was also found, but with a slightly lower correlation ($r = 0.702$; slope, 5.38; intercept, 1453.7).

The quantitative excretion patterns for free morphine, 6-AM, codeine, AC, and heroin were determined by GC-MS after administration of pure heroin containing 0–9% AC to four male subjects. Data are presented in Tables 8 and 9. Peak concentrations of free morphine, 6-AM, and codeine occurred within 1–2 h after intake. Peak concentrations of AC were not different from those of other opiates, and only free morphine and 6-AM were detected in the morning urine specimens.

The quantitative excretion patterns for all previously mentioned opiates were determined after administration of pure medical heroin. Neither AC nor codeine was detected, which demonstrates that AC is excreted in urine only when AC is present in heroin and that codeine is the main metabolite of AC.

Detection times are important markers in forensic drug testing because they indicate how long after drug administration a subject excretes a drug or a metabolite at a concentration above a specific assay cutoff (i.e., they show how long a subject tests positive for a drug) (7). The detection times by GC-MS for AC and codeine in four male subjects are shown in Tables 8 and 9. The two assigned cutoffs were 1 and 10 $\mu\text{g/L}$, respectively. Mean detection times (\pm SD) were 8 ± 4 h for AC and 23 ± 4 h for codeine.

Discussion

Our data demonstrate that AC is a good biomarker of illicit heroin use as demonstrated previously by O'Neal and Poklis (10). In their study, AC was detected in only 37% of the urine specimens, in contrast to ~86% in our study. This difference in rates could be explained by the longer period between heroin consumption and urine sampling or by the lower AC content in the heroin administered in their study.

Since 1994, when the Swiss Federal Office of Public Health started the heroin maintenance program, this is the first time that monitoring of urinary AC has been applied during such a maintenance program. No AC was

Table 7. Opiate concentrations ($\mu\text{g/L}$) in urine of patients in a heroin maintenance program.

Number	Morphine	6-AM	Codeine	AC	Heroin
1	5284	65	0	0	0
2	13 722	14 701	0	0	70
3	2646	1666	17	0	17
4	3024	6296	0	0	14
5	56 470	26 390	0	0	1006
6	39 020	39 940	0	0	693
7	12 200	17 040	0	0	511
8	4263	20	0	0	0
9	1850	156	468	0	18
10	71 060	13 710	0	0	219
11	3201	1617	0	0	18
12	6660	8791	0	0	329
13	4201	2189	0	0	40
14	641	16	0	0	0
15	6267	2644	0	0	2738
16	2205	83	0	0	0
17	523	0	0	0	0
18	9282	52	0	0	0
19	3523	3641	0	0	74
20	33 960	27 320	0	0	702
21	78 290	63 000	0	0	51 978
22	8272	10 052	0	0	1666
23	455	153	0	0	0
24	4116	2265	0	0	182
25	3681	121	0	0	0
26	760	220	0	0	0
27	2889	3836	0	0	14
28	9449	510	0	0	61
29	6654	9212	0	0	1002
30	48 970	5130	0	0	0
31	8633	951	0	0	20
32	2188	4350	0	0	219
33	8109	0	0	0	0
34	29 490	9880	0	0	26
35	14 819	4500	0	0	160
36	13 450	429	0	0	5
37	536	0	0	0	0
38	6728	0	0	0	0
39	88 090	84 720	0	0	8649
40	10 182	4874	0	0	164
41	18 056	0	0	0	0
42	2513	0	0	0	0
43	739	17	0	0	0
44	2783	0	0	0	0
Mean	14 769	8422	11	0	1604
Median	6461	1928	0	0	18
Maximum	88 090	84 720	468	0	51 978
Minimum	455	0	0	0	0
Rate, %	100	84.1	4.6	0	59.1

found in the urine of these patients, whereas concentrations of other opiates were higher than in the urine of illicit heroin users. In addition, detection times were determined for AC and codeine by parallel administration of heroin containing various percentages of AC to patients

Table 8. Measured opiate concentrations ($\mu\text{g/L}$) in urine of four patients after administration of pure heroin and heroin with 3% AC.

Patient	Time, h	Morphine	6-AM	Codeine	AC	Heroin	Volume, mL
Pure heroin							
A	0	28	0	0	ND ^a	ND	1
	1	67 070	40 980	0	0	119	14
	3	93 189	12 761	0	0	4	70
	6	7955	172	0	0	41	300 385
B	0	5834	0	0	0	2	100
	1	37 640	40 300	0	0	3340	70
	3	ND	ND	ND	ND	ND	200
	6	14 368	11	0	0	51	100 470
C	0	10 926	1409	0	0	66	200
	1	23 360	5850	0	0	129	600
	3	4978	87	0	0	0	400
	6	2332	15	0	0	0	950 2150
D	0	925	0	0	0	0	100
	1	66 950	46 810	0	0	544	30
	3	33 423	4536	0	0	37	200
	6	787	142	0	0	2	50 380
Heroin with 3% AC							
A	0	9976	0	0	0	0	25
	1	92 500	40 000	2100	224	132	35
	3	5600	590	470	8	0	600
	6	4852	142	309	2	0	570
	12	10 492	0	328	0	0	520
	24	9322	0	81	0	0	500 2250
B	0	5627	0	0	0	0	50
	1	25 120	25 180	1340	1195	1945	50
	3	ND	ND	ND	ND	ND	0
	6	6949	653	414	16	23	775
	12	29 765	18 653	366	4	1923	300
	24	2907	0	0	0	0	1200 2375
C	0	16 662	0	0	0	0	150
	1	59 290	13 900	1780	165	332	250
	3	6275	128	685	6	0	700
	6	1259	12	171	0	0	680
	12	8621	55	477	0	0	1120
	24	11 399	0	296	0	20	550 3450
D	0	2062	0	0	0	0	100
	1	37 180	27 710	1680	621	990	100
	3	618	778	297	24	34	400
	6	1192	15	86	0	0	100
	12	3742	46	579	0	0	300
	24	977	0	24	0	0	1300 2300

^a ND, not determined.**Table 9. Measured opiate concentrations ($\mu\text{g/L}$) in urine of four patients after administration of heroin with 5% and 9% AC.**

Patient	Time, h	Morphine	6-AM	Codeine	AC	Heroin	Volume, mL
Heroin with 5% AC							
A	0	7345	0	0	0	0	13
	1	94 570	45 590	45 300	329	165	30
	3	11 640	1340	1080	16	7	400
	6	11 818	119	1485	3	0	50
	12	9380	139	312	0	0	60
	24	7078	0	56	0	0	425 978
B	0	8605	0	0	0	0	40
	1	45 450	34 170	3510	2380	2022	30
	3	13 251	1097	1141	26	10	180
	6	3909	81	286	1	0	40
	12	23 366	9495	387	2	361	380
	24	6209	264	46	0	0	930 1600
C	0	14 517	317	0	0	0	400
	1	38 090	12 860	1750	330	152	280
	3	7968	777	1161	16	9	600
	6	3561	154	793	7	4	550
	12	16 361	1570	1332	0	32	1000
	24	ND ^a	ND	ND	ND	ND	720 3550
D	0	1237	0	0	0	0	100
	1	37 440	24 490	2130	567	291	100
	3	1044	70	83	1	0	200
	6	3949	69	425	0	0	200
	12	4957	82	128	0	0	500
	24	1270	41	28	0	0	220 1320
Heroin with 9% AC							
A	0	9350	191	0	0	0	20
	1	234 600	112 600	21 300	2001	575	10
	3	147 050	17 550	19 300	181	0	50
	6	13 340	710	2750	6	0	300
	12	56 160	10 900	2250	1	59	220
	24	14 417	329	134	0	0	600 1200
B	0	15 742	0	0	0	0	50
	1	29 980	25 720	4660	4870	1877	200
	3	5581	649	1163	66	4	400
	6	6440	95	937	5	0	400
	12	23 366	9495	387	1	389	250
	24	9584	319	23	0	3	450 1750
C	0	5264	397	0	0	8	550
	1	35 444	14 770	5160	1021	638	200
	3	17 627	2050	5487	89	53	400
	6	1206	15	481	1	0	1000
	12	12 343	1172	1249	1	9	1300
	24	761	33	122	0	0	1050 4500
D	0	3086	26	0	0	0	30
	1	53 390	30 880	5870	1594	367	50
	3	25 101	5884	4106	224	58	100
	6	9795	120	1176	3	0	365
	12	7556	224	192	1	0	115
	24	2898	32	25	0	0	2060

^a ND, not determined.

in the heroin maintenance program who had volunteered for the experiment. The measured detection time of 8 ± 4 h was close to that of 6-AM (7). This study also clearly demonstrates that the presence of codeine in urine could be caused by either codeine or illicit heroin consumption. However, with a measured detection time of 23 ± 4 h, codeine remains a much less specific marker of illicit heroin use than AC.

In conclusion, this study shows that, together with the detection of 6-AM in urine, AC is a suitable marker of illicit heroin use.

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