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Detection of Cannabinoids in Homicide Victims and Motor Vehicle Fatalities

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ABSTRACT: A gas chromatographic/mass spectrometric (GC/MS) procedure is described for the detection and measurement of Δ^9-tetrahydrocannabinol, 11-hydroxy-Δ^9-tetrahydrocannabinol, and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid in blood, or 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid in urine. About 50% of all homicide victims and motor vehicle drivers killed in Bexar County in 1985 were tested for the presence of cannabinoids. Of 130 homicides and 69 drivers tested, blood was analyzed primarily in all but 15 and 3 cases, respectively. In these latter cases, blood was analyzed after urine was found to be positive. Of the homicide victims, 44 (34%), and of all drivers, 19 (28%), tested were positive for one or more of the cannabinoids. As a separate group, 16 motorcycle drivers tested had 38% positive as compared with 25% of the other vehicle drivers. Ethyl alcohol was present in 55% of the drivers, and in 63% of the homicide victims. Drugs other than alcohol or cannabinoids were found in 10% of the drivers, and in 12% of the homicide victims.

KEYWORDS: toxicology, marijuana, chemical analysis

In recent years, reliable methods for the detection of cannabinoids in biological specimens have become available. Some are now extensively used in drug detection laboratories. Urine testing procedures, based on immunoassay or thin-layer chromatography, and blood procedures utilizing immunoassay are the methods most commonly used. These procedures, however, are limited for forensic science use, for the following reasons. First, they are not completely specific for the analytes, and must be confirmed by another procedure; second, they measure either one target analyte with which cross-reactivity may occur from other cannabinoids, or a composite of reactive metabolites; and thirdly, blood concentrations of cannabinoid metabolites cannot be measured.

Urine testing is further limited in significance by the fact that cannabinoid metabolites may be excreted for more than one month after cessation of use of cannabinoids, and therefore, a positive test result may have no relationship to any recent use or effects of the drug [*1*].

In contrast, the presence of Δ^9-tetrahydrocannabinol and its metabolites in blood can often be related to recent use of *Cannabis*, and may be indicative of drug effects [*2*].

The present study measured three cannabinoids in blood by a specific gas chromato-

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graphic/mass spectrometric (GC/MS) procedure to determine the incidence of use of marijuana in homicide victims and motor vehicle driver fatalities.

Methods

The procedure used for extracting Δ^9-tetrahydrocannabinol and its metabolites in blood was similar to that described by Vinson et al. [*3*] for Δ^9-tetrahydrocannabinol (Δ^9-THC) in blood. The cannabinoids were then methylated for analysis by mass spectrometry. For analysis of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (carboxy-THC) in urine, an alkaline hydrolysis step was added before extraction as with blood, and the methyl ether, methyl ester was analyzed by mass spectrometry, similar to previously described procedures [*4*].

Cannabinoids in Blood

A 3.0-mL sample of blood is placed into a 15.0-mL test tube fitted with a Teflon®-lined screw cap, along with 25 μL of internal standard (10.0-μg/mL cannabinol in absolute ethanol). Of phosphate buffer, pH 3.0, 3 mL are added, and the tubes vortexed for a few seconds. Of the extraction solvent (heptane:isoamyl alcohol, 9:1), 10 mL are then added and the tube is placed on a mechanical shaker and mixed for 5.0 min. After centrifugation to separate the layers completely, the top organic phase is placed in a separate 15.0-mL test tube to which has been added 4.0 mL of modified Claisen's alkali (3.7 g of potassium hydroxide, in 20 mL of water, and 100 mL of methanol). After mixing on a mechanical shaker for 5 min, and centrifuging, the top organic layer is aspirated to waste.

The aqueous layer is acidified with seven to nine drops of concentrated hydrochloric acid, and 10 mL of extraction solvent are added. After mixing on the mechanical shaker for 5 min, and centrifuging, the top organic layer is transferred to a 10-mL conical test tube. The organic layer is then removed by evaporation under air while maintaining the tube in a water bath set at 50 to 60°C.

To the residue, 20 μL of acetonitrile, 6 μL of trimethylanilinium hydroxide (TMAH) 25% in methanol, and 10 μL of iodomethane are added. The mixture is vortexed, then allowed to stand for 5 to 10 min before analysis by GC/MS.

Parameters for GC/MS

A Hewlett-Packard 5985A mass spectrometer was used with a Hewlett-Packard 5840A gas chromatograph fitted with a 4 ft (1.2 m) 2-mm inside diameter (ID) gas chromatographic column, packed with 3% OV101 on 80-100 Supelcoport (Supelco).

The injection temperature was 300°C, and oven temperature was programmed from 240 to 285°C at 5.0° per minute, with a run time of 10 min.

Ions monitored were as follows: Δ^9-THC 313, 328, and 245; hydroxy-THC- 313, 314, and 245; carboxy-THC 313, 357, and 372; cannabinol (internal standard), 309.

Quantitation

Working standards of 10 μg/mL were prepared for the three analytes, where 1 μl = 3.33 ng/mL, 5 μL = 16.66 ng/mL, and 25 μL = 83.33 ng/mL when these volumes are added to 3.0 mL of blank blood.

Retention times relative to the internal standard, cannabinol, and absolute retention times for all the cannabinoids tested were: Δ^9-THC—0.75 (0.6 min), cannabinol—1.0 (0.8 min); hydroxy-THC—1.375 (1.1 min), 11-nor-Δ^8-tetrahydrocannabinol-9-carboxylic acid—1.5 (1.2 min), carboxy-THC—1.675 (1.3 min), and 11-nor-H^3 Δ^9-tetrahydrocannabinol 9-carboxylic acid—1.75 (1.4 min).

Quantitations were based on relative abundances of the 313 ion for Δ^9-THC, 11-hydroxy-THC, and carboxy-THC, with the 309 ion of the internal standard, using linear regression curves set up using the three concentrations of each analyte in blood as described above. Absolute recoveries from blood averaged 76% for Δ^9-THC, 51% for hydroxy-THC, and 73% for carboxy-THC using three concentration ranges.

Internal Standards

Alternate internal standards tried included oxyphenbutazone and d^3-11-nor Δ^9-tetrahydrocannabinol-9-carboxylic acid. Oxyphenbutazone was found unsatisfactory because of difficulty in reproducibility of recoveries.

Cannabinol is the least expensive of the readily available cannabinoids, and is probably found in extremely low concentrations, if at all, in blood or urine after use of marijuana [5]. Urine and blood from 20 cases were analyzed and found positive for 1 or more of the 3 cannabinoids had no cannabinol detected. Cannabinol is hydroxylated and further oxidized in a manner similar to Δ^9-THC when given intravenously [5]. We have observed no interference from these products by this method.

Urine

For carboxy-THC in urine, the procedure described for blood was used with the following modifications: 5 mL of urine, to which has been added 50 ng/mL of cannabinol (25 μL of 10.0 μg/mL of stock solution), are hydrolyzed by adding 0.5 mL of 50% potassium hydroxide. The tubes are placed in a water bath for 10 to 15 min at 50 to 60°C.

After hydrolysis, the pH is adjusted to about 2.0 with concentrated hydrochloric acid (approximately 10 to 15 drops) before extraction, and the sample is analyzed as described above.

Other Drugs

All cases included in this study were tested also for alcohols, as well as acidic, neutral, and basic drugs. Procedures used for these consisted of GC with GC/MS for confirmation of most drugs detected [6–8]. Since virtually all of the homicide and motor vehicle fatalities were tested for other drugs and alcohol, the statistics for these agents included a larger group.

Case Studies

Study 1

Of all motor vehicle drivers killed below the age of 50 years in Bexar County in 1985, 59% were tested for blood cannabinoids. Motorcycle driver fatalities were considered separately. Drivers were selected for the cannabinoid study at random, although some selectivity occurred towards those in the younger age groups. Police accident reports were reviewed on each case to attempt to determine if the driver killed had been at fault in his fatal accident. Each of the drivers was also tested for the presence of alcohol or other drugs in the blood samples. Those in which this could not be done, for example, in delayed deaths where initial blood samples were not available, were not included in the statistics.

Study 2

Of 267 homicides occurring in Bexar County in 1985, 131 (49%) were tested for cannabinoids. The cases were selected for testing at random. Of those tested, blood was analyzed for

cannabinoids as described, except for 15 cases in which urine was tested first (and the blood was negative). Alcohol and other drugs of abuse were tested for in blood as previously described.

Blood samples were collected from the aorta and placed in 15-mL red-top (untreated) test tubes. Bloods were stored under refrigeration at 4°C upright in test tube racks until analyzed. The cannabinoids analysis was performed as soon as possible after collection, varying between 1 and 15 days. It has recently been observed that gray-top Vacutainers® (containing potassium oxalate and sodium fluoride) provides stabilization of cannabinoids in blood, and consequently, these tubes are now used routinely for blood collected for this purpose.

Results

The motor vehicle and motorcycle drivers were considered separately (Tables 1 and 2). A total of 110 motor vehicle driver fatalities occurred, of which 53 of 98 cases available for testing were tested for the presence of cannabinoids. Of these, 13 (25%) were positive for 1 or more cannabinoids. When separated into the at-fault and not-at-fault categories, the percentages of positives were essentially the same (25% for at fault, and 23% for the not at fault). By contrast, ethyl alcohol was present in 64% of the at-fault drivers tested, and only 23% of the not-at-fault drivers. Of the 13 cannabinoid positive cases, 9 also were positive for alcohol (17 of these were at fault, and 2 were not at fault).

The motorcycle drivers had a higher incidence of cannabinoid positive drivers (Table 2). Of 13, 5 (38%) of the at-fault drivers, and 1 of 3 (33%) of not-at-fault drivers were positive for cannabinoids. All 6 of those positive for cannabinoids also were positive for ethyl alcohol (average concentration 0.156%).

Of the total motor vehicle and motorcycle drivers, 66 of 120 cases tested were positive for alcohol, and there were 16 drug detections other than cannabinoids in 12 cases (see Table 3). The ages of the drivers tested for cannabinoids as compared with those not tested are shown in Table 4. Although the greatest number of positives occurred in the 20 to 29-years age group (8 of 30 or 27%), the highest percentage was in the 30 to 39-years age group (6 of 17, or 35%).

TABLE 1—*Motor vehicle drivers killed (1985).*

	At fault	Not At Fault	Total
Total	79	31	110
Tested for CBN	40	13	53
Positive	10 (25%)[a]	3 (23%)[b]	13 (25%)
Tested for alcohol	72	26	98
Positive	46 (64%)	6 (23%)	52 (53%)

[a]Seven were also positive for alcohol.
[b]Two were also positive for alcohol.

TABLE 2—*Motorcycle drivers killed (1985).*

	At Fault	Not At Fault	Total
Total	19	8	27
Tested for cannabinoids	13	3	16
Positive	5 (38%)	1 (33%)	6 (38%)[a]
Tested for alcohol	15	7	22
Positive	10 (66%)	4 (57%)	14 (64%)

[a]All six drivers were also positive for alcohol.

TABLE 3—*Alcohol and other drugs in motor vehicle and motorcycle drivers (122 cases).*

Alcohol	66 (54%)
Diazepam	4
Phenobarbital	4
Phenytoin	3
Chlordiazepoxide	1
Cocaine	1
Ephedrine	1
Meprobamate	1
Methamphetamine	1
16 drug detections in 12 cases (10%)	

TABLE 4—*Age (yrs) of tested and not tested drivers.*

Age	Total Cases	No. Tested	No. Positive for CBN (%)	Not Tested
<16	2	1 (50%)[a]	0 (0)	1
17-19	21	18 (86%)	4 (22)	3
20-29	49	30 (61%)	8 (27)	19
30-39	29	17 (57%)	6 (35)	12
40-49	12	3 (25%)	1 (33)	9
>50	24	0	0	24
Total	137	69 (59%)	19 (28)	68

[a]Percent of total cases that were tested.
[b]Percent of tested cases that were positive.

Few drivers in the 40 to 49-years age group and no drivers in the greater than 50 age groups were tested.

The homicide victims had the highest incidence of positive tests for cannabinoids (Table 5). Of 267 total victims, 130 or 49% were tested. Of these, 44 (34%) were positive for 1 or more cannabinoids. In the homicides, 151 of 241 cases tested were positive for alcohol, and there were 36 drug detections in 28 cases (other than cannabinoids) (see Table 6).

As with the motor vehicle drivers, the homicide cases were subdivided into age groups (Table 7). Of the positive cases, 61% were in the age group 20 to 29 years, while 40% of those tested in this age group were positive. The next most likely age group to have positive tests for cannabinoids was the 30 to 39-years age group. Of all positives, 25% were in this age group, and 34% of all those tested in this age group were positive.

In Table 8, the incidence of the individual cannabinoids present is shown in both the motor vehicle drivers and the homicide victims.

TABLE 5—*Homicides in Bexar County (1985).*

	Total	GSW	Other
Total	267[a]	169	98
Tested for CBNs	130 (49%)	95 (56%)	36 (37%)
Positive for CBNs	44 (34%)	33 (35%)	11 (31%)

[a]Alcohol was present in 63% of all homicides.

TABLE 6—*Alcohol and other drugs in homicides (241 cases).*

Alcohol	151 (63%)
Diazepam	10
Propoxyphene/norpropoxyphene	6
Morphine	4
Cocaine	3
Phenobarbital	3
Phenytoin	2
Methadone	2
Codeine	1
Pentazocine and tripelannamine	1
Phenmetrazine	1
Meperidine	1
Amobarbital	1
Brompheniramine and orphenadrine	1
36 drug detections in 28 cases (12%)	

TABLE 7—*Age (yrs) of tested and not tested homicides.*

Age	Total Cases	No. Tested For CBN	No. Positive	Not Tested
<16	15	2 (13%)[a]	0	13
16-19	27	17 (63%)	4 (24%)[b]	10
20-29	93	68 (73%)	27 (40%)	25
30-39	73	32 (44%)	11 (34%)	41
40-49	32	9 (28%)	2 (22%)	23
>50	27	2	0	25
Total	267	130 (49%)	44 (34%)	137

[a]Percent of total cases that were tested.
[b]Percent of tested cases that were positive.

TABLE 8—*Detection of cannabinoids in homicides and drivers.*

	Homicides (130 Tested)	Drivers (69 Tested)
No. with all three	6	2
THC and COOH only	11	9
COOH alone in blood	12	5
Urine COOH alone	15	3
Total positive for CBN	44	19

Δ^9-THC was detected in the blood of 17 of the 44 positive homicides, and 11 of the 19 cannabinoid positive motor vehicle cases. Concentrations ranged from 0.70 to 107.9 ng/mL. The incidence of hydroxy-THC was only 6 and 2 in the two groups, respectively, and concentrations found tended to be low. Concentrations ranged from 0.10 to 17.42 ng/mL, but only 2 of the cases had greater than 10 ng/mL.

The carboxy-THC was detected in the blood of the majority of positive cases. Of the 44 positive homicides, 29 had carboxy-THC detected as did 16 of the 19 positive drivers. Concentrations ranged from 2.1 to 86.7 ng/mL. Of the cases, 13 had blood concentrations greater than 25 ng/mL. Of 33 cases in which urine was screened, 18 had carboxy-THC detected in urine but not in blood. Of these, 15 were in the homicide group, and 3 were in the driver group.

Examples of cannabinoid, as well as ethyl alcohol, concentrations detected in motor vehicle fatalities or homicides are shown in Table 9. These cases were selected to show some of the extremes in variability of concentrations of cannabinoids detected. For example, in Case 1152, a high urine concentration of 637.7 ng/mL of carboxy-THC was found in a case with no cannabinoids detected in the blood, and in Case 736-85, a urine concentration of 847 ng/mL of carboxy-THC was found, with no cannabinoids detected in blood.

Discussion

In a study of 484 fatally injured drivers and pedestrians in Ontario, 16 cases had THC detected in blood. These comprised only 3.7% of the drivers and 4.2% of pedestrians, and the maximum Δ^9-THC concentration found was 5 ng/mL. The presence of other cannabi-

TABLE 9—*Blood cannabinoids, ng/mL.*

Case	Age, Race, and Sex	Δ^9-THC	OH-THC	COOH-THC	Details of Case
0797-83	18 W/M	NT	NT	NT	Ur COOH-THC—25.5 ng/mL; motorcycle driver, survived 14 days
0439-85	24 W/M	3.9	7.6	17.5	ETOH—0.179%; motorcycle driver lost control, hit pole
0736-85	25 B/M	0	0	0	Ur COOH-THC—847 ng/mL
0900-85	27 W/M	0	0	29.8	Ur COOH-THC—345.6 ng/mL; ETOH—0.07%; Mult. GSWs
1152-85	23 W/M	0	0	0	Ur COOH-THC—637.7 ng/mL; ETOH—0.23%; MVA pedestrian
1448-85	30 W/M	<1.0	1.1	17.7	Ur COOH-THC—10.6 ng/mL; ETOH—0.17%, MVA driver, at fault
1455-85	28 W/M	1.2	4.2	19.2	Ur COOH-THC—20 ng/mL; mult. stabwounds
1458-85	19 W/M	...	<0.1	2.8	Ur COOH-THC—8.9 ng/mL; GSW head, homicide
1532-85	28 W/M	5.5	0	17.5	Ur COOH-THC—31.4 ng/mL; ETOH—0.03%; motorcycle driver—hit head-on by car
1539-85	27 W/M	107.9	0	43.8	Ur COOH-THC—697 ng/mL; MV driver, not at fault
1572-85	20 W/M	0.8	0	8.8	Ur COOH-THC—312 ng/mL; stabwound, homicide

noids were not reported [*9*]. In a sequential study, this same group reported 10.9% positive for Δ^9-THC in 1394 driver and pedestrian fatalities [*10*].

In 600 drivers killed in single-vehicle crashes in North Carolina over a 3-year period, Δ^9-THC was detected in blood in 7.8% of the cases. Although the limit of the assay used was 0.4 ng/mL in blood, a cutoff concentration of 3.0 ng/mL was used to distinguish between positive and negative cases. Those positive for THC were also analyzed for carboxy-THC, but not for hydroxy-THC [*11*].

In our study, all cases in which any of the three cannabinoids could be detected and verified, using the three most characteristic ions and ion ratios for each, were considered positive. Since the carboxy-THC metabolite is present in blood for a much longer period than Δ^9-THC or hydroxy-THC, it is not surprising that our study had much higher incidences of positive cases than previous ones. In the drivers, 28% overall (motor vehicle—25%, and motorcycle—38%) were positive for one or more cannabinoids, and in the homicides, 34% were positive. If the eighteen cases in which urine only was positive are subtracted our figures become 23% for drivers and 22% for homicides. If detection of Δ^9-THC in blood is used as the criterion, our study population had 13.0% of homicides and 15.9% of the drivers positive (see Table 8).

No attempts were made to relate the use of marijuana to the circumstances of death in this study. However, the presence and ratios of the individual cannabinoids in blood can indicate time since use of marijuana, and if certain criteria are met, whether or not some pharmacological effect would have been present.

For example, Δ^9-THC and hydroxy-THC are approximately equally pharmacologically active. In general, subjective effects, and objective correlates of effects such as pulse rate, coincide with both of these in the blood [*2,12*]. The hydroxy metabolite is very transient, and its presence in any concentration would be suggestive of effects. The 9-carboxy metabolite is totally inactive pharmacologically. However, it increases in blood as the Δ^9-THC is decreasing, and persists for a much longer period of time at relatively high concentrations [*13,14*]. Therefore, the ratio between the two can be used to indicate recent or more remote use of marijuana. If blood THC concentrations are equal to or higher than the carboxy-THC, death would have occurred within approximately 30 min of smoking, and near peak effects would have occurred [*10*]. However, if carboxy-THC alone is detected in blood or urine, no statements concerning effects can be made.

Using disposition and metabolism data from previous studies of marijuana smoking subjects, a table can be constructed to provide guidelines for interpretation of cannabinoids in blood (See Table 10) [*2,13-15*].

Most studies indicate maximum effects at approximately 1 h after smoking, and waning to absent at 3 to 4 h. Therefore, carboxy-THC/THC ratios of greater than four, may indicate a time point past pharmacological effects [*11*].

TABLE 10—Δ^9-THC metabolites in blood after smoking, %.

	½ h	1 h	2 h	3 h	24 h
Δ^9-THC	45[a]	15	27	20	5
OH-THC	5	10	5	0	0
COOH-THC	45	50	63	75	80
Other polar acids	5	25	5	5	15
COOH-THC/THC	1	2	3	4	7

[a]Relative proportions of each cannabinoid expected to be present [*12*].

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