Detection of cannabinoids in serum of vehicle drivers after smoking cannabis in coffee shops

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1. Introduction

Attempts to correlate plasma concentrations of THC with levels of psychomotor impairment have met with only limited success. Neither experimental nor epidemiological approaches to the cannabis question have yet provided definitive answers (Daldrup et al., 1987). The absolute THC levels associated with impairments are highly variable. One estimate is that only levels of 25 ng/mL or higher would be definitely associated with impairment in every case (Hollister, 1988). McBay concluded in one of his recent reviews: "the major problem is the lack of control studies of the frequency of occurrences and the concentrations of cannabinoids in the general driving population. Such studies have been reported for alcohol, but have not been reported for marijuana because of the infinitely more complex problem of obtaining blood specimens from the general driving population and the difficulties and expense of the analyses".

The special geographic situation in our country allows us to obtain blood specimens from such a general driving population and to study the influence of cannabis smoking. It is well known that it is possible to legally buy small amounts of cannabis products in The Netherlands especially in so called "Coffee Shops". Not so in Germany. So many of german cannabis users regularly travel to The Netherlands to smoke hashish in one of these numerous Coffee Shops along the frontier. Very often several grams of hashish are smoked. Afterwards they drive back home and very often try to smuggle hashish or other drugs, too. So regularly checkpoints are installed by the german police. If there is any suspicion of an offence under the influence of drugs, blood specimens are taken.

2. Toxicological Analysis

The blood specimens are brought to our laboratory by courier. Immediately 2-3 mL of serum are separated and few milligrams of sodium fluoride are added. These samples are stored at -18°C for confirmation analysis. Aliquot parts of the remaining serum are precipitated with acetone. 0.1 mL of the supernatant clear solution is used for immunoassays for cannabinoids, opiates, amphetamines and cocaine metabolites.. 10 µL saturated sodium chloride solution is added to improve conductivity. Serum standards are used to calibrate the assays. All positive results are confirmed either by GC/MS or for cocaine by HPLC/DAD using the separated serum specimens.

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Positive cannabinoid results are confirmed by GC/MS after solid phase extraction (column: AMCHRO, endcapped, 100 mg, 1 mL) at pH of 4. We use a deuterium labelled internal standard:

Sample Preparation:
1. 1 mL serum + internal standard + acetic acid (pH= 4)
2. Apply to a (conditioned) extraction column
3. Wash with 1 mL acetic acid
4. Wash with 1 mL acetonitrile (40% in water)
5. Extract twice with 0.75 mL acetonitrile (100%)
6. Dry under nitrogen

The extract is methylated by a common derivatization method:

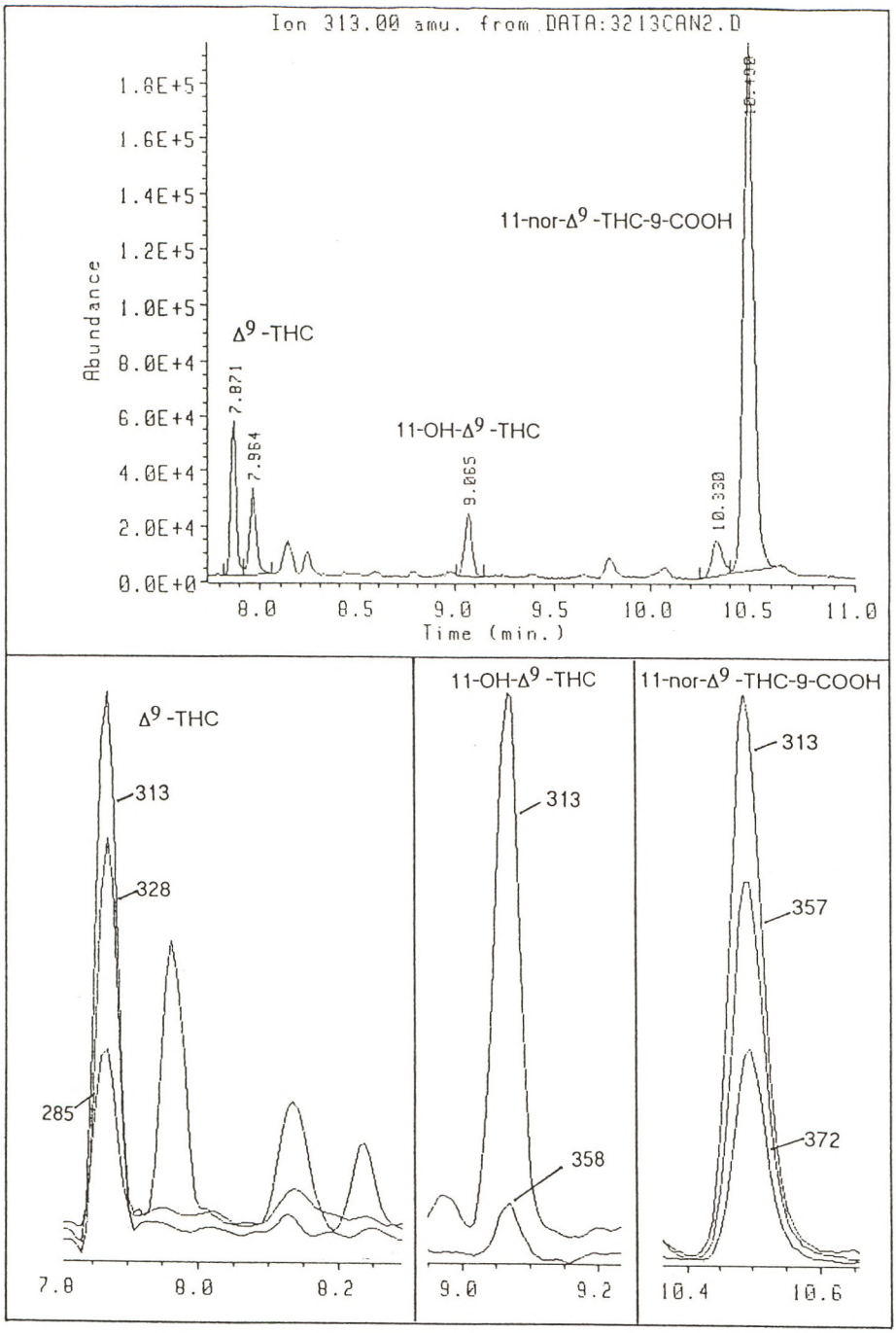
Derivatization method:
1. Add 200 µL TMAH (20% in water) : DMSO (1:20) and vortex
2. Let stand for 2 min. at room temperature
3. Add 50 µL methyl iodide and let stand 15 min. at room temperature
4. Add 200 µL HCl (0.1 mol/L) and extract with1 mL iso-octane
5. Dry under nitrogen
6. Reconstitute with 20 µL ethyl acetate.

1 to 2 µL are injected for GC/MS analysis. Retention time and selective mass fragments are used to detect the different cannabinoids:

Mass Spectrometric Parameters:
Selective ion monitor for:
THC: 285, 313, 328 (RT: 7.87 min.)
11-OH-THC: 313, 358 (RT: 9.07 min.)
11-nor-THC-COOH: 313, 357, 372 (RT: 10.49 min.)
D3-11-nor-THC-COOH: 316, 360, 375 (RT: 10.49 min.)

Figure 1 shows an example of a typical chromatogram. The serum specimen belongs to a young man, who admitted to have smoked 1 g of hashish about one hour before being arrested by the police. We have found THC in a concentration of 19 ng/mL, Hydroxy-THC in a concentration of about 4 ng/mL and free THC acid in a concentration of 70 ng/mL.

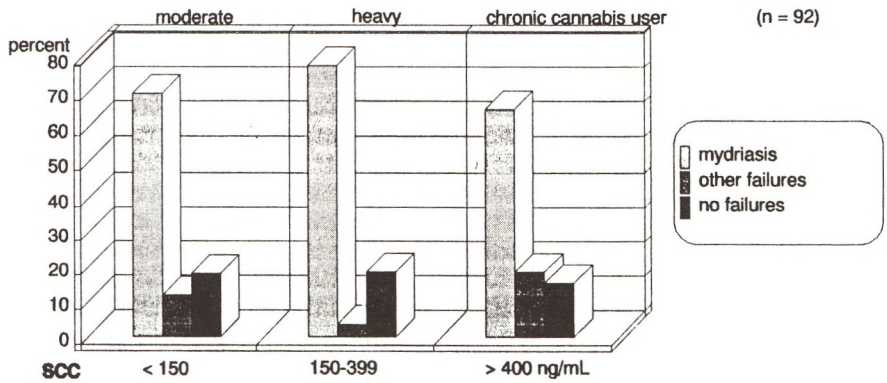
Figure 1: Typical chromatogram of an authentic serum specimen with 19 ng/mL THC, 4 ng/mL 11-OH-THC and 70 ng/mL free 11nor-THC-9-COOH



3. Results

From all the samples we had to analyse during the first six month of this year, we selected those positive for cannabinoids and negative for the other drugs. This gave us the opportunity to correlate impairments and test failures reported by the police and/or the doctor with serum concentrations of THC and its metabolites. We have a collective of 92 persons, 87 men and 5 women. Because of its very long biological half-life of about 8 days, free and conjugated THC-carbonic acid accumulate in serum, if hashish is smoked regularly (Daldrup et al., 1988). This gave us the opportunity to divide our collective into three groups: Moderate (n=33), heavy (n=27) and chronic (n=32) cannabis users.

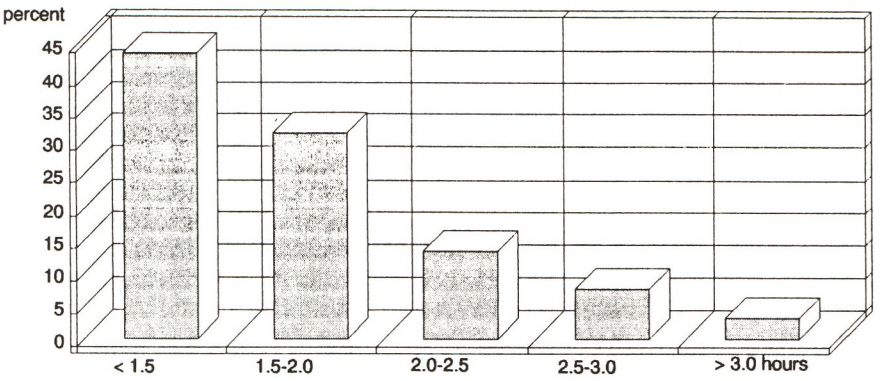
Figure 2: Moderate (n=33), heavy (n=27) or chronic (n=32) cannabis use versus failures reported by the doctor.



A person is called moderate cannabis user if the serum cannabinoid concentration, the "SCC", as determined by immunoassay, is below 150 ng/mL. If a SCC between 150 and 400 ng/mL is observed, the person is called heavy user. If the SCC exceeds 400 ng/mL chronic use is assumed. This is of course our personal definition, however we find it very suitable for the daily work in forensic toxicology. First we wanted to know, if differences could be observed among these three groups about the failures reported by the doctor. There are minor differences. The predominate symptom in all three groups were the dilation of pupils. For this test a common pocket-lamp was used. About 60 to 75% of all examined persons had a mydriasis not responding to illumination. Other impairments like delayed intellectual performance were seen more frequently in the group of chronic users (Figure 2).

You may now ask, how this frequency of impairments can be explained. All persons had been asked by the police when they had smoked hashish. 51 of them gave an answer.

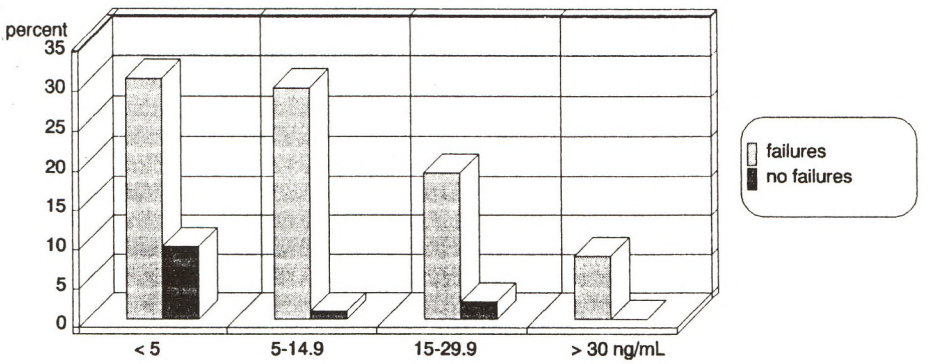
Figure 3: Time between cannabis smoking and incident (n=51)



We see the great majority had smoked cannabis shortly before being checked by the police. In nearly 75% of the cases this time was below 2 hours (Figure 3). As cannabis effects last at least 2 hours, it is easily understood, why such a frequency of impairments was found.

The most important guideline to measure cannabis effects is the serum THC concentration. We see in Figure 4 that even if the THC concentration is below 5 ng/mL failures are reported very often.

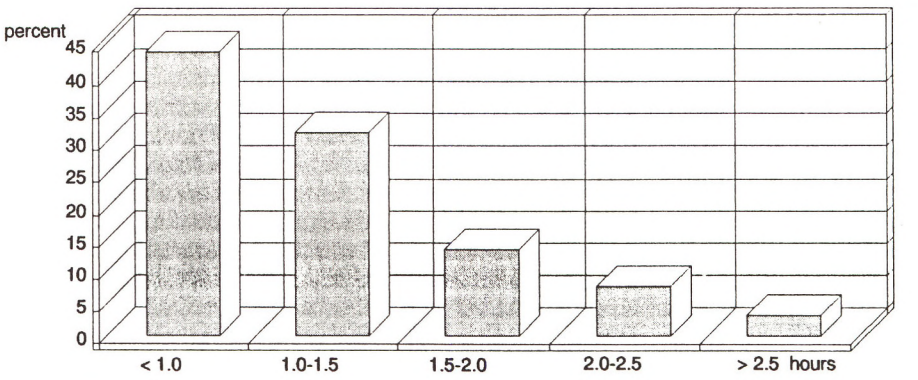
Figure 4: THC-levels (C_0) in serum versus failing of tests (n=92)



This result corresponds with results published in literature. But it must however be admitted a quarter of the persons in this group showed no impairments. Not so in the groups with THC concentrations above 5 ng/mL. Here we had no failures in isolated cases only. And finally if the THC concentration was above 30 ng/mL failures seem to be inevitable. Similar results have been observed in studies with volunteers.

Some of you may be amazed about the number of forensic cases with high THC concentrations. The reason is very simple (Figure 5):

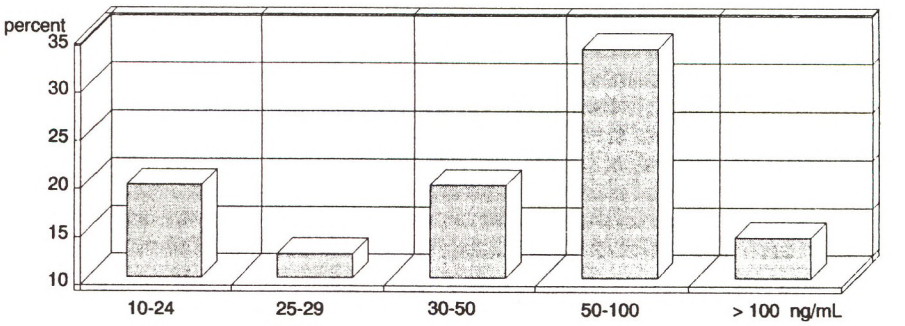
Figure 5: Time between incident and taking the blood specimen (n=89)



There is a good cooperation between the police and the examining doctors. So the time between incident and the blood sampling is generally below one or one and a half hour. This time is short enough to detect THC in serum in high concentrations despite its half-life of only about 30 to 40 min. during the beta-phase of elimination.

Using pharmacokinetic data published for THC we calculated the possible serum THC - concentration C_t for the time of incident in all these

Figure 6: THC-levels (C_t) at the time of incident (t) (n=55)

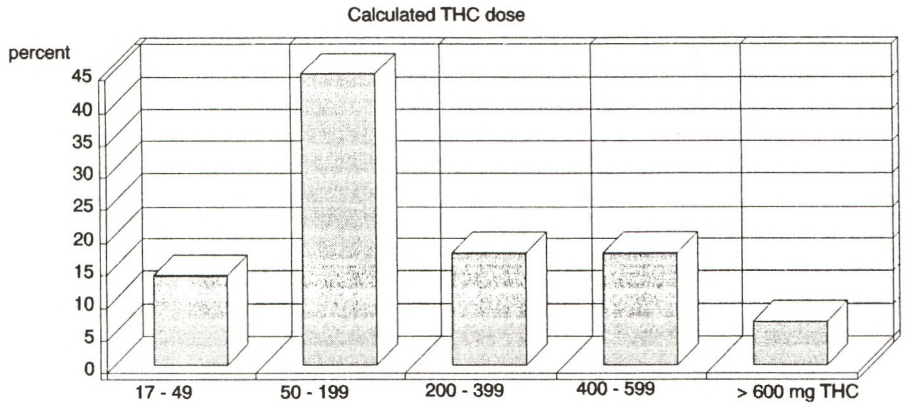


cases (n=55) with a C_0 exceeding 5 ng/mL. Concentrations below 5 ng/mL

were rejected because we did not know, whether we still were in the β-phase of elimination or already in the γ-phase with a considerably longer half-life of THC. We used a volume of distribution of ten litres per kilogram body weight, a half-life of 40 min. and applied kinetic law of first order kinetics. Estimating THC obeys this simple pharmacokinetic model, we observed that the majority of case had serum THC concentrations at the time of incident exceeding 25 to 30 ng /mL, concentrations associated according to many authors with impairments in every case.

With the data we have, it is also possible to calculate the amount of THC in the body immediately after smoking (Figure 7).

Figure 7: Amount of THC in the body after smoking (n=29)



I admit the results of all these calculations are not correct in every case and therefore should only be used with great care in forensic toxicology. But on the other hand to my opinion such calculations are necessary to get on in research especially in the field of driving under influence of cannabinoids. Figure 7 shows us that the THC concentrations we found in the serum can very often be explained only if we admit that several hundreds of mg of THC were consumed and absorbed via the lungs. These amounts are clearly higher than those used in studies with volunteers. Perhaps this difference between reality and controlled studies with volunteers is a mean reason why controlled studies have often failed to demonstrate a negative influence of THC on driving abilities. The THC doses smoked in these studies were too low and did not at all reflect reality.

Table 1 summarizes all our results. We had 92 persons who had smoked hashish. Analysis for other drugs were negative in all cases. 33 were called moderate, 27 heavy and 32 chronic cannabis users. The mean serum cannabinoid concentrations, the SCC, were around 80, 240 or 700 ng/mL respectively for each of these groups.

Very interesting are the mean serum concentrations of THC and its active metabolite Hydroxy-THC. The group of chronic cannabis users showed by far the highest concentrations for both substances. However, the ratio Hydroxy-THC to THC was nearly identical in all three groups. We found

a ratio of around 0.6 to 0.8. These ratios are considerably higher than the ratio of 0.1 observed in controlled studies. This is a further proof that such studies do not reflect reality.

Table 1: Summary of the results of toxicological analysis

users	n	SCC ng/mL	THC ng/mL	THC-OH ng/mL	11 nor-THC-COOH (free) ng/mL	ratio THC-OH/ THC	ratio SCC/ THC-COOH
all	92	341.8	11.3	8.1	88.3	0.72	3.87
moderate	33	82.9	3.5	2.7	24.8	0.77	3.34
heavy	27	238.9	9.6	6.1	67.0	0.64	3.57
chronic	32	695.5	19.9	15.4	171.6	0.79	4.05

The ratio in the last column is proportional to the ratio conjugated and free THC-carbonic acid in serum. It can be seen that chronic users have the highest and moderate users have the lowest ratio. This result can be explained if we admit a slightly longer serum elimination half-life for the conjugated THC-carbonic acid than for the free THC-carbonic acid.

I hope, our study has shown that the use of low serum THC concentrations for the estimation of impairment after moderate cannabis smoking remains difficult to be established. Not so, I believe, if chronic cannabis use can be proved and/or if high levels of THC plus Hydroxy-THC are present in serum. Further studies with an improved number of cases will show us if we are on the right way.

4. References

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