Perspectives for the detection of cannabis in breath

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Abstract
Cannabis is the most frequently detected drug in many epidemiological studies on drugs and driving in Europe: 10% (median of 10 studies) of injured drivers and 7.6% of killed drivers are positive. The possibility of detecting cannabis in breath would greatly simplify controls in drivers, both for epidemiological studies and law enforcement.
Only a few studies (all performed in the seventies and eighties) on cannabis in breath exist (see table).

Author, year	Enrichment method	Detection	Detection limit (ng THC)	Duration of detection (min)
McCarthy, 1971	Filter paper or tissue	Colorimetric Fast Blue B	10	120
Hauck, 1974	Absorption by petroleum ether	TLC	1	CBN & THC 8 CBD 14
Valentine, 1979	Polyethylene foam wafer or ethanol cryogenic trap	LC-MS	(5)	240 ?
Manolis, 1983	Syringe containing Tenax GC	GC-MS	0.25	10-12

Many studies were hampered by problems with the analytical technique. One wonders whether new detection techniques could increase the detection time. In order to have a sensitive and reliable detection of cannabis in breath, several aspects will have to be resolved: choice of the target molecule, sampling and enrichment method, avoidance of contamination by ambient air, sensitivity of detection, both for screening and confirmation, …

Introduction
In recent years, the interest for roadside drug testing has significantly increased (1). The preference of police officers goes to a device with the ease-of-use and speed of a breathalyser (2). Cannabis is the most frequently detected drug in many epidemiological studies on drugs and driving in Europe: 10% (median of 10 studies) of injured drivers and 7.6% of killed drivers (median of 9 studies) are positive (3). The possibility of detecting cannabis in breath

would greatly simplify controls in drivers, both for epidemiological studies and law enforcement. The aim of this contribution is to review the existing literature on the detection of cannabis components in breath.

Methods

A literature review was performed on Medline and Analytical Abstracts. In addition, information was searched on the Internet.

Results

Only five studies were found. Most of the literature dates from the 70s and the 80s. McCarty and Van Zyl (4) developed a ’breathalyser’ based on a colorimetric test. The subject was required to breathe onto a paper (filter paper or tissue) freshly dampened with aqueous Fast B salt (1 % w/v). The reagent was stated to have a sensitivity of 10 ng. The colour response ranged from bright orange-pink to deep orange-pink tinged with mauve up to 15 minutes after smoking. The colour was obtainable up to two hours after one typical cannabis cigarette. Colour formation was sometimes not instantaneous. The authors remarked that South African cannabis is potent and that the signal could be weaker elsewhere. They observed that South African toasted cigarettes (that do not contain cannabis) and smoked tobacco also gave a positive response with this test. Nutmeg gave a positive result as well, but spices and peppermints gave only the faintest response. They also described colour tests to confirm the results.

Hauck and Moll (5) developed a thin layer chromatographic method using DC-alu-Fertigfolien A 1500 LS 254 Kieselgel impregnated with dimethylformamide and developed with cyclohexane. They detected tetrahydrocannabinol (THC) with Fast Blue B 1%. At that time (1974), it was not possible to detect cannabis components in blood nor urine! They could detect cannabidiol (CBD) and THC from 1 ng of cannabis. They experimented with cigarettes containing 50 mg of hashish and captured the smoke in a flask containing 50 mL of petroleum ether. The solvent was concentrated to a few microlitres by evaporation and applied on TLC plates. THC, CBD and cannabinol (CBN) could still be detected from 125 µL of the petroleum ether (1/400 of the total volume). Absorption on activated charcoal and elution with petroleum ether did not give good results. A volunteer experiment was also carried out. The subject smoked a cigarette containing 250 mg of hashish during a period of 10 - 13 minutes. He was asked to fill a 1-litre plastic bag and the breath was passed through 50 mL of petroleum ether. CBD could be detected up to 8 minutes after smoking. When 3 litre of expired air was blown through the petroleum ether, CBN and THC could be detected for 8 minutes after smoking cessation, while CBD was detectable for 14 minutes. They also used a gas chromatograph with flame ionisation detection and electron capture detection, but some components present in petroleum ether had retention times close to that of some cannabis components and interfered with the low concentrations.

Valentine et al. (6) used an LC-MS method with deuterated internal standards. THC was collected on a polyethylene foam wafer (3 cm in diameter and 0.25 cm thick, held approximately 1.5 cm in front of the subject’s lips) which was positioned in front of the expired air stream using a modified face mask or with a cryogenic trap containing 10 mL of ethanol. The foam wafer was sonicated for 30 minutes, while submerged in methanol in a silylated beaker. Internal standard (1.6 µg of trideuterated THC) was added. After evaporation, the residue was reconstituted in 300 µL heptane and analysed by LC-MS. With the cryogenic tube, the inside was washed with 1 mL of ethanol and this, together with the 10 mL of ethanol, was evaporated. The further manipulation was similar to that for the foam wafer. The subject smoked one marijuana cigarette containing 10.8 mg of THC and was asked to breath for 1 minute through either apparatus at different time points after cessation

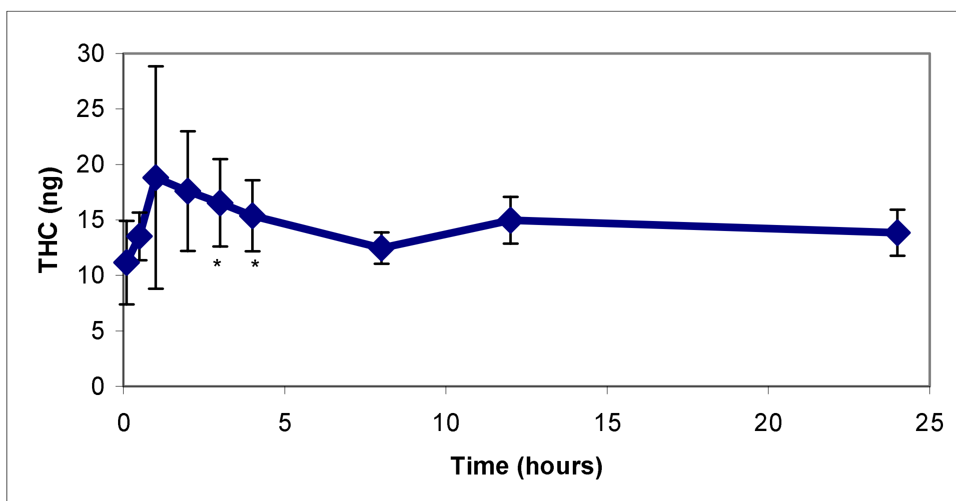

Figure 1: Nanograms of THC found in human breath before and following marihuana smoking using a polyethylene foam wafer (figure drawn from the data in reference 6).The vertical lines represent the standard deviation. The high value (11.2 ng) before smoking is due to the contribution of THC present in the internal standard. The values were compared to the baseline values with a one sided paired T-test, and the points marked * were statistically significantly (respectively P = 0.047 and 0.03 after 3 and 4 hours) different from the baseline value.

of smoking. Unfortunately, the internal standard contained trace amounts of THC and the results are difficult to interpret (figure 1). Although the authors claim (based on the mean of 11.2 ng and a standard error of the mean of 1.5 ng) that their method was able to detect THC up to 24 hours following smoking (with the exception of the sample taken at 8 hours), statistical analysis with a one sided T-test shows significant results only after 3 and 4 hours. With the cryogenic breath trap, the levels were higher at 0.25 hours than at baseline (P = 0.04; only one time point was studied). The cryogenic breath trap seemed more efficient than the foam wafer, but it was less portable. The authors also wondered whether saliva (containing THC) did not contaminate the breath.

Manolis et al. (7) compared seven different absorbents for THC entrapment. Care was taken to avoid salival contamination. They used detection by GC-MS. Five of the tested sorbents [30 mg Tenax-GC 60 80 mesh, with thermal desorption (T1) or extracted with 2 x 2 mL methanol (T2), 100 mg Tenax GC extracted with methanol (T3), bubbling tube apparatus with ethanol as extraction system (A) and bubbling tube apparatus with 0.75 N KOH in ethanol as extraction solvent and back-extraction into 105% isoamyl alcohol heptane (K)] yielded detectable THC concentrations at 10 – 12 minutes post-smoking of two cigarettes (containing the equivalent of 150 µg THC/kg body weight). The absolute quantities of THC recovered ranged from 0.4 to 8.1 ng. Corrected for recovery, this corresponded to 3.1 - 20.8 ng or 0.18 – 5.7 ng/L breath. The lower detectable limit was 250 pg of THC or 0.02 ng/L (in the case of a sample volume of 30 L of breath). The best recovery was obtained with method T1, but it suffered from the disadvantage of requiring the subject to use considerable pressure to blow through the narrow bore tubing containing the Tenax-GC. The decay of breath THC was much more rapid than blood THC.

The authors concluded that the detection of breath THC was probably due to that emanating from the surface of the mouth and respiratory system.

More recently, in May 1998, an article in "The Australian" was mentioned on the Internet (8). It announced that a breath test unit to screen drivers for cannabis has been developed to prototype stage by doctors Ron Parsons and Zenon Mejglo, honorary fellows at the department of chemistry of the University of Tasmania in Hobart. The heart of the cannabis breath test device was a sensor disc, the exact chemical composition of which is a secret shared only by Drs Parsons and Mejglo. When an affected driver blows into a mouthpiece, chemicals detecting cannabis turn the sensor disc red. Light hits the disc, is reflected and measured by a photo diode. With any colour in it, the disc will show proportionately less light and provide a reading. No more information could be obtained on this device. Doctor Parsons and Mejglo both died in 1998 and no further development occurs on this project.
It seems that some other projects were funded (personal communication by some colleagues), but no results could be found in the literature.

Discussion

Most work on the analysis of cannabis in breath was performed several years ago. There were problems with the analytical methods, and the maximal detection time was 4 hours.
In order to have a sensitive and reliable detection of cannabis in breath, several aspects will have to be resolved.
The target molecule could be THC, CBD and/or CBN, but it could also headspace volatiles (9). However, one should be sure that the target molecule is found only in marijuana or hashish and the choice of a non cannabinoid compound as marker could make the results less easy to defend legally.
A good sampling mechanism, which allows to collect the components present in one or several litres of breath without the need for blowing too hard or too long, will have to be developed. Moreover, the sample size should be standardised and reproducible (10).
Due to the very low levels of cannabis components in breath, preconcentration should be used. The three main methods currently utilised for preconcentration are chemical interaction, adsorptive binding and cold trapping (11), but each method has some disadvantages. Solid-phase microextraction (SPME) has also been used for some volatiles like ethanol (detection limit 200 ng/L) acetone (detection limit 300 ng/L) and isoprene (detection limit 75 ng/L) in human breath (11). One of the disadvantages of SPME is that the absolute recovery is rather low.
With one exception, the experimentation with breath tests for cannabis has been performed more than 15 years ago. Lack of sensitivity and specificity or problems with the analytical technique hampered the researchers. At this time it is difficult to make an estimation of the needed sensitivity: should it be 1 picogram or 1 femtogram? For a roadside breathalyser, the technique should be portable. Some prototypes of immunoassays developed by STC technologies (12) using 'Up converting phosphor technology (UPT)' are capable of detecting 1 ng of THC. On-site analysis by ion mobility spectrometry is also a possibility. In the lab, GC-MS-MS, GC-NICI-MS and LC-MS-MS seem to be good candidates to obtain the maximal sensitivity and specificity.
Moreover, if the assay is very sensitive, care should be taken to avoid all external contamination, e.g. by performing the breath test in an environment that is free of cannabis smoke and avoiding that ambient air passes through the sampling device. Room air THC levels were 10 – 30 ng/L in a small (2.1 m x 2.5 m x 2.4 m) room where 2 subjects smoked 4 cigarettes with the door open and 1540 – 2960 ng/L in the same conditions but with the door closed (13).
In conclusion, it is hard to predict whether cannabis breathalysers will ever be practicable. On the one hand, increasing sensitivity of the analytical techniques could render them possible, but on the other hand, very little is known and much work still needs to be done on sampling

and enrichment, specificity and avoidance of contamination. However, work progresses in other uses of breath, e.g. in the diagnosis of several diseases (10, 14), and it may be possible that breakthroughs in this area also contribute to the development of a cannabis-breathalyser.

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