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IN DOPING ANALYSIS  
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U. MARECK-ENGELKE, H. GEYER, W. SCHÄNZER:  
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## **Tetrahydrocannabinol (THC) in dope control**

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### **Abstract**

In January 1999 the IOC decided to conduct tests for cannabinoids at the Olympic Games. A concentration in urine of 11-nor- $\Delta$ -9-tetrahydrocannabinol-9-carboxylic acid higher than 15 ng/ml is prohibited.

The standard operating procedure developed at the laboratory in Cologne for confirmation and quantification of the tetrahydrocannabinol metabolite is presented in here as well as the statistical evaluation of the urine samples, which were analyzed by GC/MS during 1998 in the Cologne laboratory.

From 7421 urine samples, 36 (= 0.5 %) contained more than 15 ng/ml tetrahydrocannabinol-metabolite. Only one sample originates from a female (teakwondo), the remainder from male athletes. The most frequently concerned federations are squash (15%), baseball (12%) and water skiing (8%). These federations have not banned cannabinoids. 20 samples were taken at competitions, the remaining 16 are out of competition controls.

The statements of the different federations regarding the abuse of tetrahydrocannabinol are in fact nonuniform.

### **Introduction**

Cannabinoids belong to the class of drugs subject to certain restrictions. Where the rules of a responsible authority so provide, tests will be conducted for cannabinoids (e.g. Marijuana, Hashish). A cut-off limit of 15 ng/ml for the main metabolite 11-nor- $\Delta$ -9-tetrahydrocannabinol-9-carboxylic acid has been added to the list in order to avoid passive smoking consideration. Prohibition of cannabinoids depend on behalf of the single federation.

Due to the fact, that consumption of tetrahydrocannabinol increases the readiness of risk, federations of high risk sports like FIS (international skiing federation) and FISA (international motor sports federation) banned cannabinoids to protect the athlete.

Until now only few more federations agree in banning this drug.

## Analytical method

The main metabolite of tetrahydrocannabinol is 11-nor- $\Delta$ -9-tetrahydrocannabinol-9-carboxylic acid (fig 1), 50-70 % is excreted as glucuronide, the rest as free form.

### *First registration of the tetrahydrocannabinol-metabolite*

The screening method for anabolic steroids (1,2) includes also a first registration of the tetrahydrocannabinol-metabolite.

The steroid conjugates of 2 or 4 ml urine (depending on the density) are deconjugated by direct enzymatic hydrolysis with  $\beta$ -glucuronidase and separated with tert.-butylmethylether. After derivatisation with MSTFA/ $\text{NH}_4\text{J}$ /ethanethiol the TMS-derivatives are analyzed by GC/MS in SIM (single ion monitoring) mode.

15 ng/ml 11-nor- $\Delta$ -9-tetrahydrocannabinol-9-carboxylic acid is added also to a mixture of metabolites from anabolic steroids (standard metabolite) which is analyzed by GC/MS with each batch of prepared urine samples. In this way a first estimation of the amount of THC-metabolite in suspicious urine sample is possible.

### *Standard operating procedure (SOP) for confirmation of the tetrahydrocannabinol-metabolite*

The sample preparation of the confirmation method is similar to the screening method (fig 3). As additional internal standard 15 ng/ml (+)-11-nor-9-carboxy- $\Delta^9$ -tetrahydrocannabinol-**D9** is added, the same amount as the cut off limit.

Most important fact of the GC/MS-parameters (tab 1) is the short temperature programm. One analytical run takes only 15 minutes and is very time effective.

The retention times of internal standard and tetrahydrocannabinol-metabolite for the described GC/MS-parameters lies between 4 and 5 minutes. The internal standard is eluted before the tetrahydrocannabinol-metabolite.

Three ions, each from tetrahydrocannabinol-metabolite and deuterated tetrahydrocannabinol-metabolite (internal standard), are used for selected ion analysis (tab 2).

The relative abundance of any of the ions shall not differ by more than five per cent (absolute) or twenty per cent (relative), whichever is greater, positive control urine or suspicious urine sample.

The order of injection into the analytical instrument is according to the IOC-recommendations:

- negative control urine
- sample being confirmed
- negative control urine
- positive control urine

|                            |  |
|----------------------------|--|
| GC/MS                      | HP 5890 II / HP 5971A                                |
| column                     | 17m Ultra 1 (OV1), 0.2 mm id, 0.11 µm film thickness |
| carrier gas                | 1ml helium at 180°C, split 1:10                      |
| GC temperature program     | 200°C, 15°C per min, 300°C (5 min)                   |
| injection port temperature | 300°C  |
| transferline temperature   | 320°C  |

Tab 1: Standard Operating Procedure (SOP)  
Confirmation of THC-metabolite 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid  
GC/MS-parameters

| m/z | fragment ions THC-metabolite           |
|-----|--|
| 488 | molecular ion (M <sup>+</sup> )        |
| 473 | M <sup>+</sup> - 15 (CH <sub>3</sub> ) |
| 371 |  |

| m/z | fragment ions D9-THC-metabolite (ISTD) |
|-----|--|
| 497 | molecular ion (M <sup>+</sup> )        |
| 479 | M <sup>+</sup> - 15 (CH <sub>3</sub> ) |
| 380 |  |

Tab 2: Standard Operating Procedure (SOP)  
Confirmation of THC-metabolite 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid  
Table of ion masses used for selected ion analysis

*Standard operating procedure (SOP) for quantification of the tetrahydrocannabinol-metabolite*

To calculate the concentration of the tetrahydrocannabinol-metabolite in both samples, the suspicious sample and the spiked control urine are prepared three times according to the above described method (SOP for confirmation of the tetrahydrocannabinol metabolite). Each sample is injected twice. The control urine is spiked with the expected amount of tetrahydrocannabinol-metabolite.

For each sample the ratio between tetrahydrocannabinol-metabolite (m/z: 371) and internal standard (m/z: 380) is calculated. By comparison of these ratios between sample being confirmed and positive control urine a calculation of the concentration of the tetrahydrocannabinol-metabolite is possible (tab 3).

Bacterial activity may lead to a reduction of the tetrahydrocannabinol-metabolite concentration in the urine sample (fig 4). This fact is very important for performance of the B-analysis.

| #                             | Standard urine<br>ratio (m/z) 371/380 |             | #                 | Suspicious sample<br>ratio (m/z) 371/380 |             |
|-------------------------------|---------------------------------------|-------------|-------------------|--|-------------|
|                               |                                       | <i>mean</i> |                   |  | <i>mean</i> |
| I (1.injection)               | 1.79                                  |             | I (1.injection)   | 2.23                                     |             |
| I (2.injection)               | 1.92                                  | 1.86        | I (2.injection)   | 2.32                                     | 2.28        |
| II (1.injection)              | 1.80                                  |             | II (1.injection)  | 2.10                                     |             |
| II (2.injection)              | 1.81                                  | 1.81        | II (2.injection)  | 2.27                                     | 2.19        |
| III (1.injection)             | 1.74                                  |             | III (1.injection) | 2.33                                     |             |
| III (2.injection)             | 1.69                                  | 1.72        | III (2.injection) | 2.28                                     | 2.31        |
| <i>mean</i>                   |                                       | 1.79        | <i>mean</i>       |  | 2.26        |
| <b>cTCH-metab. 25.2 ng/ml</b> |                                       |             |                   |  |             |

Tab 3: Standard Operating Procedure (SOP)  
Quantification of the THC-metabolite (example)  
Standard urine spiked with 20 ng/ml THC-metabolite  
Internal standard for each urine sample: 15 ng/ml D9-THC-metabolite

### Statistical evaluation

In 1998, 7421 urine samples were investigated from national and international federations. 36 urine samples (= 0.5%) contained more than 15 ng/ml tetrahydrocannabinol-metabolite (tab 4). This is the IOC recommended cut off limit which had been added to the list of prohibited substances in order to avoid passive smoking consideration.

Only one of the 36 samples originates from a female (teakwondo), the rest from male athletes. The most frequently concerned federations are squash (15%), baseball (12%) and water skiing (8%). These federations have not banned cannabinoids. 20 samples were taken at competitions, the remaining 16 are out of competition controls.

Due to the detection of banned cannabinoids in 14 urine samples, the positive results were reported to the appropriate federations.

| Discipline    | Number of samples in 1998 | THC-metabolite > 15 ng/ml |     |             |                    |
|---------------|---------------------------|---------------------------|-----|-------------|--------------------|
|               |                           | Number                    | %   | Competition | Out of competition |
| Baseball      | 43                        | 5                         | 12  | 4           | 1                  |
| Weightlifting | 884                       | 3                         | 0,3 | -           | 3                  |
| Soccer        | 891                       | 3                         | 0,3 | 3           | -                  |
| Wrestling     | 68                        | 3                         | 4,4 | 2           | 1                  |
| Skiing        | 158                       | 3                         | 1,9 | 3           | -                  |
| Sailing       | 53                        | 2                         | 3,8 | 2           | -                  |
| Squash        | 13                        | 2                         | 15  | -           | 2                  |
| Basketball    | 135                       | 1                         | 0,7 | -           | 1                  |
| Icehockey     | 73                        | 1                         | 1,4 | -           | 1                  |
| Fencing       | 111                       | 1                         | 0,9 | 1           | -                  |
| Handball      | 172                       | 1                         | 0,6 | -           | 1                  |
| Judo          | 210                       | 1                         | 0,5 | 1           | -                  |
| Canoeing      | 339                       | 1                         | 0,3 | 1           | -                  |
| Athletics     | 1157                      | 1                         | 0,1 | -           | 1                  |
| Swimming      | 309                       | 1                         | 0,3 | -           | 1                  |
| Teakwondo     | 19                        | 1                         | 5,3 | -           | 1                  |
| Gymnastics    | 84                        | 1                         | 1,2 | -           | 1                  |
| Volleyball    | 65                        | 1                         | 1,5 | 1           | -                  |
| Water skiing  | 12                        | 1                         | 8,3 | -           | 1                  |
| Unknown       | -                         | 3                         | -   | 2           | 1                  |

Tab 4: Urine samples containing more than 15 ng/ml THC-metabolite in 1998 (laboratory Cologne)

## Statement of international federations to cannabinoids

The "Directory of Anti-Doping Regulations of International Sport Federations" (3) gives a first overall view. The doping regulations of 60 international federations are presented.

Most of the international federations adopt the regulations of the IOC. Cannabinoids belong to the class of drugs subject to certain restrictions. Each single federation has to decide about a ban of cannabinoids and probably fix a cut off limit. In case of a positive urine sample the respective federation is asked for a statement concerning cannabinoids.

Some federations have their own regulations. For high risk sports like motor cycling or motor sports cannabinoids are restrictively banned. WPBSA and FIS additionally fixed a cut off limit in order to avoid passive smoking consideration.

For federations with own regulations and a missing statement to cannabinoids the conclusion can be drawn, that the use of cannabinoids is not restricted.

| kind of sport     | international federation   | remark                       |
|-------------------|--|------------------------------|
| basketball        | FIBA (Federation International de Basketball)  | 2*                           |
| billard / snooker | WPBSA<br>(World Professional Billiards and Snooker Association)  | 1*<br>cut off limit 50 ng/ml |
| archery           | FITA (Federation Internationale de Tir a L'Arc)  | 1*                           |
| curling           | WCF (World Curling Federation)   | 1*                           |
| soccer            | FIFA (Federation International de Football Association)<br>UEFA (Union of European Football Association) | 2*                           |
| weightlifting     | IWF (International Weightlifting Federation)   | 2*                           |
| pentathlon        | UIPMB (Union Internationale de Pentathlon Moderne et Biathlon)   | 1*                           |
| motor cycling     | FIM (Federation Internationale Motocycliste)   | 1*                           |
| motor sport       | FISA (Federation Internationale Du Sport Automobile)   | 1*                           |
| skiing            | FIS (Federation Internationale de Ski)   | 1*<br>cut off limit 15 ng/ml |
| gymnastics        | FIG (Federation Internationale de Gymnastique)   | 2*                           |

Tab 5: International federations banning cannabinoids

1\*Ban of cannabinoids according to own regulation

2\*Doping regulation of IOC;

Ban of cannabinoids according to declaration of the federation

## Statement of umbrella organizations to cannabinoids

The austrian BSO (Bundes-Sport-Organisation): For positive cases from competition and out of competition controls punishment of 2 years is intended.

The Italian Federation FMSI (Federations Medico Sportiva Italiana): Sanction of reported tetrahydrocannabinol-cases.

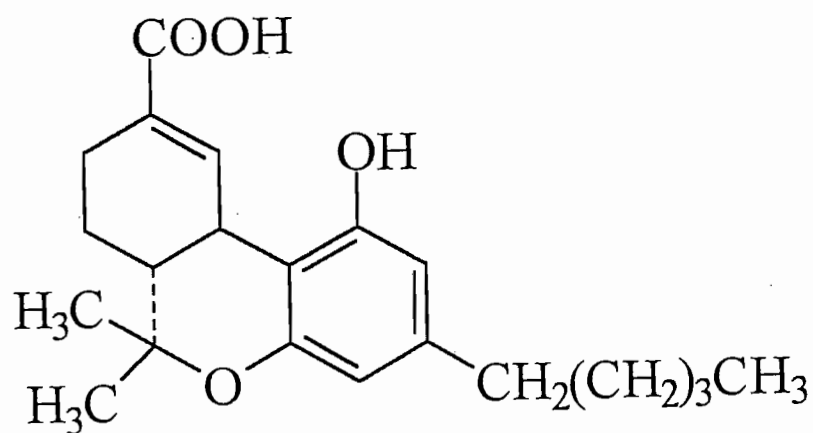
The German ADK (Anti-Doping-Kommission): At this time the federations have to give statements concerning cannabinoids. The inquiry it is not yet finished. In general the national federations agree with the international federations.

Until now urines with carboxy-THC-concentrations higher than 15 ng/ml are reported but not yet punished.

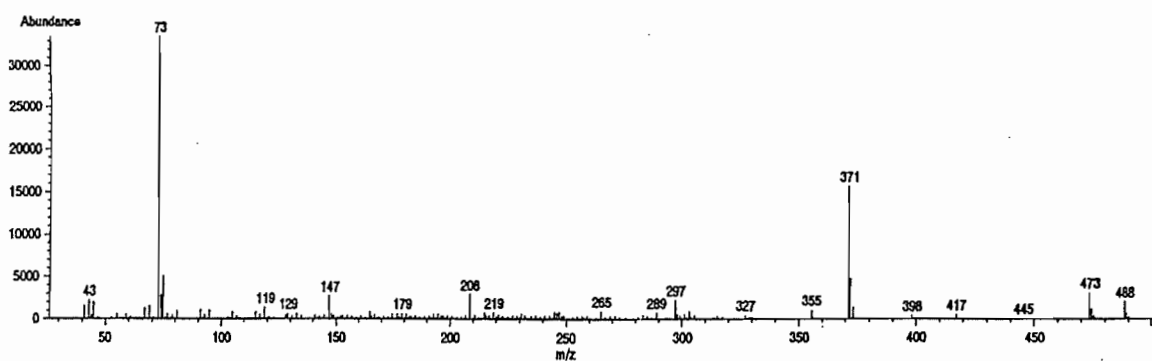
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- (2) Geyer, H., Schänzer, W., Mareck-Engelke, U., Nolteernsting, E., Opfermann, G.: Screening Procedure for Anabolic Steroids - The Control of the Hydrolysis with Deuterated Androsterone Glucuronide and Studies with Direct Hydrolysis. In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (5). Sport und Buch Strauß, Köln (1997) 99-101.
- (3) Directory of Anti-Doping Regulations of International Sport Federations (prepared by the United Kingdom Sports Council in association with the IOC)  
United Kingdom Sports Council, Ethics & Anti-Doping Directorate, Walkden House, 10 Melton Street, London, NW1 2EB, England.





**Fig 1: Structure of 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid**



**Fig 2: EI mass spectrum of the trimethylsilyl derivative of 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid**

**Standard Operating Procedure (SOP)**  
**Confirmation of the tetrahydrocannabinol metabolite**  
**11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid**

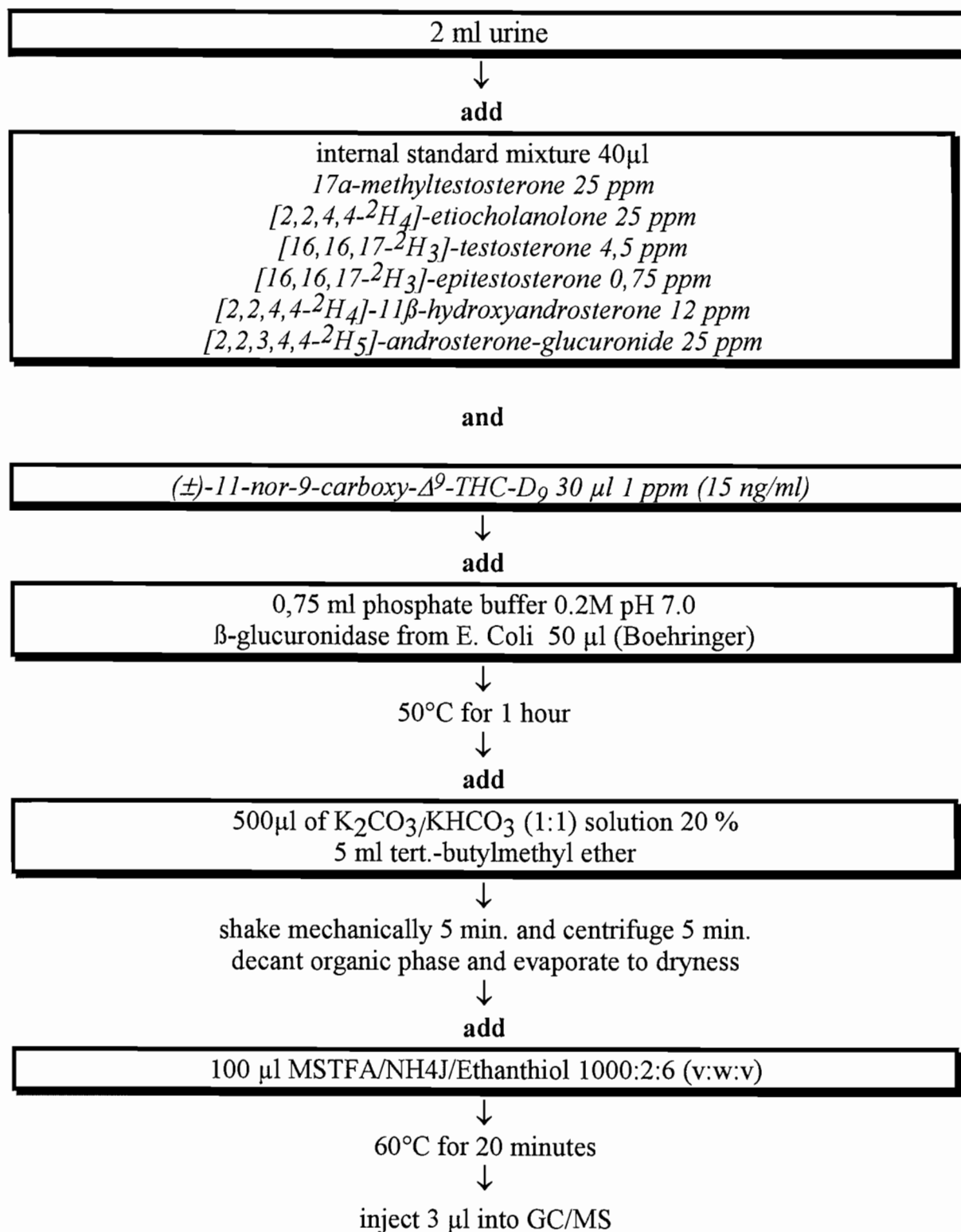
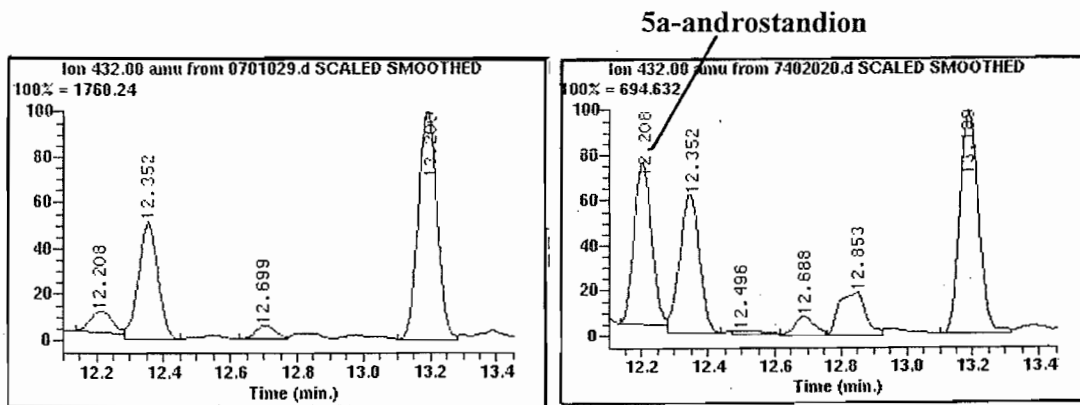


Fig 3: Standard Operating Procedure - Confirmation of tetrahydrocannabinol-metabolite



|                  | <u>A-sample</u> | <u>B-sample</u> |
|------------------|-----------------|-----------------|
| pH               | <b>5.5</b>      | <b>8.3</b>      |
| density          | 1.0126          | 1.0135          |
| date of analysis | 21.01.1999      | 19.02.1999      |
| cTHC (ng/ml)     | <b>40</b>       | <b>25</b>       |

**Fig 4: Influence of bacterial activity on the concentration of 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid**