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DRIVERS DRUG-TESTING  
IN BULGARIA: 2012.

TESTIRANJE VOZAČA NA SREDSTVA  
ZLOUPOTREBE U BUGARSKOJ 2012.

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*Key words*

Drivers drug testing; drugs of abuse; oral  
fluid testing; confirming laboratory  
analyses.

*Ključne reči*

testiranje vozača na sredstva zloupotrebe,  
sredstva zloupotrebe, testiranje salive,  
potvrđne laboratorijske analize

*Abbreviations*

THC –  $\Delta^9$ - tetrahydrocannabinol  
(cannabis); OPI – opiates; COC –  
cocaine; AMP – amphetamine; MET –  
methamphetamine; MTD – methadone;  
MDMA – 3,4-methylenedioxy-N-methyl-  
amphetamine; BZD – benzodiazepines;  
TCA – tricyclic antidepressants; BAR –  
barbiturates; 6-MAM – 6-monoacetyl-  
morphine; SPE – solid phase extraction;  
BAC – blood alcohol concentration.

*Abstract*

In the study we described the results of 57 drivers' samples (blood and/or urine) subjected to confirming laboratory analysis for presence of drugs of abuse during 2012 in Bulgaria. The aim of the research was to follow the current protocol for drivers testing in Bulgaria as well as to comment the first analytical results after initiation of roadside oral drug testing. The critical analysis of the pre-analytical stage led to the conclusion that some changes in the national regulation protocol for alcohol and drug testing of drivers are required as: i) the urine should be used as a confirming sample in the laboratory; ii) more information about the roadside testing results is needed to be submitted with samples. The analytical data from the laboratory testing present the distribution of the drivers as age, sex and group / individual compound of psychoactive drugs. The results confirm the sociological information for drugs of abuse in the country – the target group is the young population (18-25 years old) and the most used drugs are cannabis (THC) and amphetamine. The comparison of the laboratory results with these of roadside testing shows very good correlation of the results for oral fluid / blood (urine), but in some cases there are differences (false positive results for saliva or false negative results in case of more than one drug presented).

*INTRODUCTION*

The usage of psychoactive compounds by drivers is a problem in modern world which increases the number of crashes and traffic accidents. Recent studies show that blood, urine or oral fluid samples are positive for psychoactive compounds of approximately 1-12% of general driving population, 8-51% - of injured drivers and 6-35% - of killed drivers.<sup>(1-3)</sup>

Roadside driver drug check in Bulgaria starts in 2011 when Traffic Police initiates usage of on-site oral fluid testing. Before implementation of saliva tests, only suspected

drivers were subjected to blood analyses for drugs of abuse and psychoactive medicines. The regulation protocol in Bulgaria which sets alcohol and drugs testing of drivers is published in 1980<sup>(4)</sup>, revised in 2001<sup>(5)</sup> but it is not changed in the sampling and analytical part of the protocol. However, 30 years later the situation on the road and drugs used are completely different. The regulation protocol for drivers drug testing in Bulgaria needs revision in the sampling and analytical part for alcohol<sup>(6)</sup> as well as for drugs of abuse. Nowadays, according to the last addition of the Regulation 30/2001<sup>(5)</sup> in 2006, the laboratory of Military Medical Academy – Sofia is the confirming laboratory for

the whole country, equipped and capable to analyze and confirm drugs of abuse in samples according to the international recommendations (TIAFT, SOT).

In the present study we summarize the results on blood and urine testing of drivers, submitted for confirming analyses by the Police in 2012. We should emphasize that according to the national Regulation 30/2001<sup>(5)</sup>, the drivers may refuse medical check and blood testing. In this case, the result of preliminary roadside test is valid for administrative trial. However, we reviewed only the results from case samples from laboratory analysis, not all positive roadside cases.

## MATERIALS AND METHODS

### *Samples.*

During 2012 samples from 57 persons were subjected to the instrumental analysis for confirmation of drugs presence. For 9 cases we received blood samples only, in 8 cases – urine sample, and in 40 cases – both blood and urine samples. The samples were sent to the laboratory by the Police. Thirty five of the samples were from preliminary tested drivers and were positive for drugs of abuse - roadside oral fluid test (Dräger Drug Test 5000 and Dräger Drug Check). According to the current Regulation, the most blood samples consist of 40 mL blood without any preservative or anticoagulant. When the blood drawn was done in Military Medical Academy heparin-containing (green-cap) vacuum tubes were used (2 x 7 mL). All samples were stored and transported to the laboratory at 4 °C.

### *Analytical procedures.*

First, initial testing for presence of drugs of abuse in the urine samples was performed using GabControl<sup>®</sup> immunotests (GabMed GmbH, Germany) for OPI, COC, AMP, MET, THC, MTD, MDMA, BZD, TCA, BAR. As a second step general screening of urine and blood (5 mL of urine; 1 mL of blood serum/plasma, respectively) was performed using basic liquid-liquid extraction (ethylacetate) and evaporation under nitrogen (up to 50 µL) as pre-concentration step following GC-MS analyses on BP-35ms column (Trace GC equipped with DSQ single quadruple MS detector). The identification of the compounds was based on MS-spectral library search (NIST, PMW, DD 2011) and RT-table of analytical standards (Alltech, USA).

The confirming analysis for THC was performed analyzing its metabolite –  $\Delta^9$ -THC-COOH. The urine was hydrolyzed with KOH, while the blood serum/plasma was subjected to enzymatic hydrolysis using  $\beta$ -glucuronidase/arylsulfatase (Merck, Germany). Next SPE on Strata X-C (Phenomenex, USA) was performed and BSTFA derivatization prior to GC-MS analyses. The GC-MS analyses was performed in SCAN/SIM mode (m/z 371, 473, 488), LOD 200 ng/mL.

The identification of 6-MAM in the samples was performed after SPE on Strata Screen C (Phenomenex, USA), derivatization (BSTFA) and GC-MS analyses in SCAN/SIM mode (m/z 287, 399, 400).

The corresponding deuterated drugs were used as internal standards (Alltech, USA) when quantification was necessary.

The identification and quantification of the BZD was performed using HPLC-UV.

## RESULTS AND DISCUSSION

### *Pre-analytical stage.*

According to the current practice for alcohol and drug testing of drivers in Bulgaria test for drug of abuse is performed when a suspicious behavior of the driver is observed and the person refuses roadside alcohol testing. The same procedure is also applied for participants in traffic accidents. The Traffic Police in Bulgaria is equipped with oral fluid testing devices Dräger Drug Test 5000 and Dräger Drug Check. When the preliminary test is positive or the preliminary test is not available on site, the tested person has to pass medical check in the nearest Emergency Center or hospital. During the medical check, the medical doctor describes any observable symptoms of psychoactive drugs use as well as if any other drugs were prescribed to the person. Then a blood sample (40 mL, no preservative and anticoagulant added) is drawn from the person. The blood sample is subjected for drugs of abuse analysis.

Regarding the preliminary stage of drug testing we have many objections. First at all, a lots of information about roadside testing is missing. The following data usually should accompany testing protocol: i) initial reasons for testing; ii) the result of roadside alcohol testing; iii) preliminary drugs of abuse testing – positive (for which drugs), negative (not tested, negative or the person refuses testing); iv) when the preliminary test was performed. For all 57 cases' samples (52 males, 5 females) only 14 were accompanied with pre-analytical information (25%).

Next is the medical check. The testing protocol for alcohol includes different neuro-physiological symptoms as nystagmus, ataxia, size of pupils, psycho-emotional status, etc. which are not included in the „Psychoactive drugs” section of the protocol. It leads to additional loss of information, which is quite important for the final forensic interpretation of the results.

The most important pre-analytical part of the study is the sampling. However, the sampling procedure is accepted in 1980 and it is not revised in sampling section up to now. In the general toxicological screening we usually prefer urine sample (easier analytical protocol and better detection window of the drugs) which is not included as sample in the current protocol. For initial screening of blood and consequent confirmation analysis we need 10 mL of blood, preferably with anticoagulant (heparin). These considerations are included in the current project for revision of the testing procedure in Bulgaria.

### *Analytical results.*

Laboratory analysis starts with preliminary immunotest of urine sample (if available), followed by GC-MS screening analysis. When the results from immunotest and/or from instrumental screening indicate presence of psychoactive drug(s) - a confirming analysis is performed.

The age distribution of the tested persons is presented on Fig. 1. The major group is the youngest (18 to 25 years old; 23 persons or 40%) and decreasing distribution is observed

for other age groups as can be expected. This result corresponds to the other drug-related studies in the country. According to the annual reports on the problems related to drugs and drug addictions in Bulgaria – 2011(7,8), 38.9% (110 000) of university students in Bulgaria declared an experience (at least one) with drugs of abuse. However, it is interesting to note the number of positive results in next groups. As it is shown on the Fig. 1, there is a significant number of positive results in other groups which confirms the necessity of testing all age groups, not only for the young drivers.

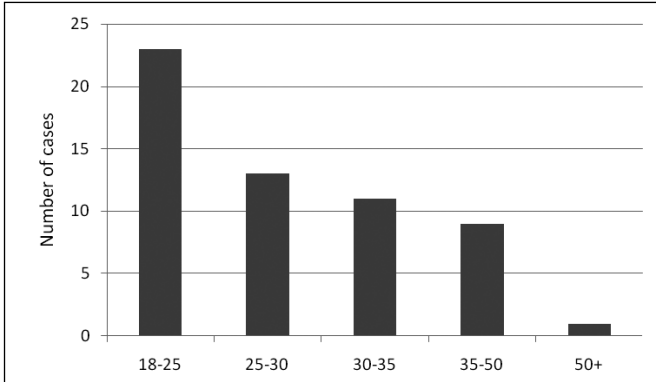


Fig. 1. Age distribution of drivers tested for drugs of abuse.

The type of psychoactive compounds according to the result obtained during laboratory testing is shown on Fig. 2. The highest result corresponds to use of cannabis (THC) – 27 cases (47%), followed by amphetamine – 14 cases (25%). It corresponds also to the results of sociological surveys in 2011 - 37% of asked respondents declared smoking of cannabis, 15% - of synthetic stimulants. There is one case of designer's drug use - pyrovalerone.

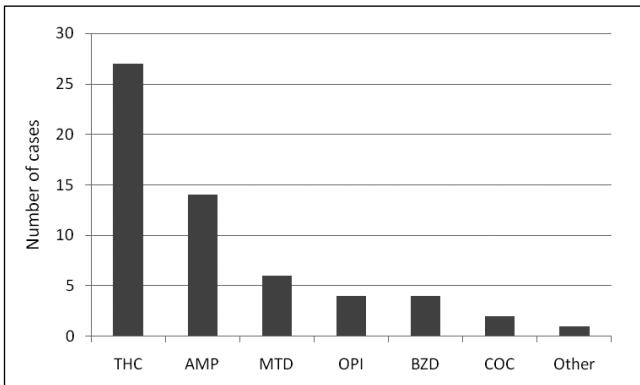


Fig. 2. Type of drugs found in the samples.

In our opinion there is another problem related to the high number of THC-positive samples in drivers. The analytical protocol for cannabis detection refers to detection of the main but not pharmacologically active metabolite of the highly lipophilic THC –  $\Delta^9$ -THC-COOH, with a long detection period in plasma / urine<sup>(9)</sup>. In this respect the result of roadside saliva test or laboratory analysis is positive even in persons without influencing of the behavior. However, the Bulgarian law prohibit usage of drugs of abuse (zero tolerance), as a difference by the alcohol impairment (limit of 0.5 g/L BAC).

There were 12 cases of combined usage of psychoactive drugs. It is interesting that 9 of them are THC-based combinations. The lower number of alcohol-based combinations is not indicative, because the roadside alcohol testing is widely performed in Bulgaria. If the driver is positive for alcohol on roadside alcohol testing, usually second test for drugs of abuse is not performed. That is why the number of alcohol-based combinations is not real. Usually, the roadside drug testing is performed when the breath alcohol test is negative, but the person shows some signs of drug usage.

As it was mentioned in 57 cases we had preliminary information about roadside oral fluid testing of 14 cases. Seven of them were with different analytical results. Other 21 samples were designated as „positive”, but 7 of them were negative in laboratory testing. The results presented show high discrepancy according to the preliminary testing which can be consequence from improper usage of the testing device and/or incorrect reading and description of the analytical result. As it is an important part of the pre-analytical step more efforts to be done are required at this stage. It is especially important when the tested person refuses blood test and then only the preliminary result is valuable for administrative purposes.

The best correlation between oral fluid testing results and from the next laboratory analysis was found for THC confirmation – all positive cases in oral fluid testing were confirmed in the laboratory. The most problematic drug was found to be amphetamine in urine (immunotest) – false positive result or cross-interference by metamphetamine.

### CONCLUSION

On the basis of the results obtained, several points as conclusion are defined:

1. Revision in the sampling protocol is required. i) The urine sample should be stated as a second sample for drug analyses. It allows easier sample preparation with a high concentration ratio and detection of drugs on broader time-scale. Usage of urine sample alone is not recommended because the origin of the sample is questionable (no DNA and other specific markers). When is possible the saliva sample could be also send to the laboratory. ii) The volume of blood should be reduced from 40 mL to 10 mL and anticoagulant (heparin) must be added. iii) Completion of the on-site questionnaire should be introduced in order to obtain more detailed information about driver's status, roadside tests as well as leading to better chain-of-custody registration.
2. Oral fluid testing is a good initial screening procedure for usage of illegal drugs of abuse. However, the results from the study presented indicate that the confirming laboratory analysis must be obligatory. A number of submitted samples differs in results from the roadside saliva test. Most probably it is an artificial error and more important is the positive result, but there was a number of false positive tests (if the sampling protocol is adequate!).

3. The distribution of the drugs among the drivers is similar to the distribution in the country. Cannabis (THC) and synthetic stimulants are the most widely used drugs by drivers. The data for designer drugs are not adequate because this type of compounds is not detected using oral fluid testing devices and sometimes – in laboratory.

Table 1. Combined usage of psychoactive drugs.

Drugs	Number of cases
THC + AMP	4
THC + MTD	2
THC + OPI	1
THC + MET	1
THC + AMP + MDMA	1
MTD + BZD + Pyrovalerone	1

### Apstrakt

U prikazanoj studiji prikazani su rezultati analiza 57 uzoraka krvi i/ili urina vozača koji su poslani na potvrdnu analizu na prisustvo sredstava zloupotrebe u toku 2012. godine u Bugarskoj. Cilj ovog istraživanja bio je da prati važeći protokol za testiranje vozača u Bugarskoj kao i da diskutuje prve analitičke rezultate posle testiranja pljuvačke na terenu. Kritička analiza preanalitičke faze dovodi do zaključka da su potrebne izvesne izmene u nacionalnom regulatornom protokolu za testiranje vozača na prisustvo alkohola i sredstava zloupotrebe u sledećem: 1) urin treba da bude potvrdni uzorak za laboratorijsku analizu, 2) više informacija o testiranju na terenu potrebno je dostaviti sa uzorkom. Analitički rezultati dobijeni nakon laboratorijskog testiranja prikazani su prema godinama vozača, polu i grupi / pojedinačnom psihoaktivnom jedinjenju. Rezultati prikazuju sociološku informaciju o zloupotrebi droga u Bugarskoj – ciljna grupa je mlada populacija (18-25 godina), a najčešće korišćena droga je kanabis (THC) i amfetamin. Poređenje laboratorijskih rezultata sa onima nakon testiranja na terenu pokazuje veoma dobru korelaciju između pljuvačke i krvi odn. urina, mada u izvesnim slučajevima postoje razlike (lažno pozitivni rezultati za salivu ili lažno negativni rezultati u slučaju kada je više od jedne supstance prisutno).

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