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CONFIRMATORY AND COMPARATIVE DRUG ANALYSIS OF ORAL FLUID AND BLOOD SAMPLES FROM DRIVERS SUSPECTED OF DUID

POTVRDNA I KOMPARATIVNA ANALIZA UZORAKA PLJUVAČKE I KRVI NA PRISUSTVO PSIHOAKTIVNIH SUPSTANCI KOD VOZAČA

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Key words

Driving under the influence of drugs; Drugs of abuse; Oral fluid and blood testing; Confirmatory and comparative analysis

Ključne reči

vožnja pod dejstvom psihoaktivnih supstanci, sredstva zloupotrebe, pljuvačka i testiranje krvi, potvrdna i komparativna analiza

Abstract

Confirmatory drug analysis in drivers' blood samples was performed in Bulgaria (2013-2014). The study was based on 241 samples collected from drivers suspected of driving under influence of drugs (DUID). Data obtained were compared with the results from preliminary oral fluid (OF) testing. The survey showed that the young people aged 18-25 are the main group of drugged drivers. Analytical data from laboratory blood testing indicated that the most common drug of abuse is tetrahydrocannabinol. The combined illicit drug usage by drivers was also frequently detected. Additional comparative analytical study of 50 used on-site devices and their test strips revealed that in-lab analysis of strips as a dried matrix is a suitable alternative to blood testing. The results showed that it is necessary to continue monitoring of both matrices (OF and blood) for the purposes of DUID control and forensic toxicology.

INTRODUCTION

Nowadays, drugs of abuse can be analyzed in different biological matrices which are characterized by some advantages and disadvantages. In cases of driving under the influence of drugs (DUID), blood is considered to be the best matrix for analysis [(1)]. It provides unique benefits over other matrices with respect to the extensive amount of published reference data for drug concentrations and to the pharmacologically interpretive value (ensures better interpretation of exposure degree and drug-likely effects)[(2)]. Blood also possesses disadvantages as invasive collection by medical personnel and short detection time for many drugs [(2-3)].

Recently oral fluid (OF) became a widely used sample for preliminary screening of drugs of abuse, particularly in on-site (roadside) testing [(1, 4-7)]. It is considered as an alternative biological matrix that manifests some advantages over blood and urine specimens in terms of readily available sample; easy, non-invasive and painless collection; supervised collection without privacy invasion; lower risk of adulteration; shorter detection window and better correlation with drug effect (indicates recent usage); active product assessment (not metabolites) [(8-9)]. The roadside OF testing is currently based on immunoassay using various technical devices [(5, 7-8, 10-11)]. However, in case of positive OF screen-

ing result, still blood remains a preferable matrix for confirmative drug analysis [12].

In Bulgaria a „*per se*" legislation (Zero Tolerance law) for drugs of abuse used by drivers exists. Zero Tolerance law makes it illegal to drive with any measurable amount of specified (illicit) drugs in the body. This facilitates implementation of DUID legislation, since the prosecution does not have to prove that driver was impaired. The use of drugs of abuse has to be evidenced by results from technical means (roadside OF devices) and / or from laboratory blood tests.

Current practice in Bulgaria (since 2011) for drug testing of drivers with suspicious behavior consists in OF testing by on-site devices Dräger Drug Test 1200 / 5000. In case of positive result or refusal of such inspection, driver must go through medical examination during which the physician describes observable symptoms of illicit drugs use (behavior, general mental and somatic condition of the driver, any symptoms of abstinence, etc.) as well as if any other medicines were prescribed to the person. A blood sample is taken and submitted for subsequent analysis. In case of driver's denial to give a blood sample for drug testing, results based on Dräger Drug Test are accepted.

In the present work, the results from confirmatory drug analysis in blood and their comparison with data obtained from preliminary on-site OF drug testing are reported. The survey was performed in Bulgaria within two-year period (2013-2014) using samples from drivers suspected of DUID. A comparison between results received from roadside testing devices and in-lab analysis of their test strips is also presented.

MATERIALS AND METHODS

Materials

All reagents were of analytical grade and all solvents used - of chromatographic grade. Sodium hydroxide (NaOH), phosphoric acid (H_3PO_4), anhydrous sodium sulfate (Na_2SO_4), dichloromethane (CH_2Cl_2), methanol (MeOH), ethanol (EtOH), *i*-propanol (*i*-PrOH), acetonitrile (MeCN), ethylacetate (EtOAc), methyl *tert*-butyl ether (MTBE), ammonium hydroxide (NH_4OH), pentafluoropropionic anhydride (PFPA), N,O-*bis* (trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA), N-*tert*-butyldimethylsilyl-N-methyltrifluoroacetamide with 1% *tert*-butyldimethylchlorosilane (MTBSTFA) were purchased from Sigma–Aldrich (Germany) and acetic acid (AcOH), sodium acetate (AcONa), disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), sodium hydrogen carbonate ($NaHCO_3$), *n*-hexane (*n*-C_6H_{14}), toluene, β-glucuronidase/aryl sulfatase (β-GLU; 30 U/L: pH 3.8, 38 °C) – from Merck (Germany). In all experiments deionized water (18.2 MΩ•cm) was used.

Sample collection

Blood samples (n = 61 for 2013 and n = 180 for 2014) were collected from drivers suspected of DUID. Collection was based on existing traffic control testing procedure in Bulgaria. On-site testing devices Dräger Drug Test 1200 / 5000 cartridges (n = 50, 2014) collected from roadside OF testing were provided by relevant authorities for confirmatory testing and were accompanied by whole blood sample from the tested person. All technical devices were stored at 4 ºC until analyzed. Roadside testing and collection of whole blood samples were completed within one hour.

Laboratory analysis

Plasma samples were analyzed after suitable protein precipitation (PPT), liquid-liquid extraction (LLE) or solid phase extraction (SPE), depending on the preliminary results available, by gas chromatography with mass spectrometric detection (GC-MS; Agilent 7890B GC system interfaced with an Agilent 5977A mass selective detector) with library search (NIST, PMWTOX3N, DD2011) for 11-nor-9-carboxy-delta-9-tetrahydrocannabinol(THC-COOH), amphetamine (AMP), methamphetamine (MET), cocaine (COC) and opiates (OPI) to confirm a positive or negative usage of these drugs of abuse.

THC-COOH in blood was detected using following sample-preparation protocol: 1 mL plasma sample was hydrolyzed for two hours at 55 °C after addition of 100 µL 100 mM acetate buffer solution pH 4.5 and 50 µL β-GLU. After hydrolysis sample was cooled down to room temperature (RT), and 2 mL ice-cold MeCN:MeOH = 85:15 solution was added. Then mixed-mode cartridge Strata Screen C (Phenomenex, USA) was used to perform SPE according to manufacturer's instructions. Dried extract was derivatized by addition of 30 µL EtOAc and 30 µL MTBSTFA (70 °C, 20 min) and sample obtained (1 µL) was injected for GC-MS analysis. GC was fitted with HP-5ms capillary column (30 m x 0.25 mm x 0.25 µm). The column temperature program was as follows: 60 °C (2 min), 60-150 °C (30 °C/min), 150 °C (1 min), 150-280 °C (8 °C/min), 280 °C (15 min). Electron ionization MS operating conditions were 230 °C ion source temperature and 70 eV electron energy. Mass-spectral detector was operating using full scan mode in the range of 50-650 m/z. The retention time (Rt) for THC-COOH as MTBSTFA derivate (m/z 413, 515, 572) was 26.8 min. Limit of detection (LOD) was found to be 10 ng/mL.

AMP and MET were assayed using LLE as follows: 1 mL plasma was extracted with 4 mL EtOAc in basic media (1 mL 1 M NaOH) after PPT with 2 mL MeCN. After vortex-mixing and centrifugation (3 000 rpm / 3 min), organic layer was evaporated to dryness under N_2 and derivatized with 250 µL toluene: MeCN = 95:5 and 25 µL PFPA. Mixture was heated at 45 °C for 10 min, next sample was cooled to RT and 1 mL 5% $NaHCO_3$ solution was added to remove (neutralize) acidic by-products. 1 µL of toluene phase was then injected into GC-MS. GC-MS oven temperature was: 50 °C (2 min), 50-170 °C (15 °C/min), 170 °C (1 min), 170-280 °C (20 °C/min), 280 °C (7 min). The Rt for PFP derivatives of AMP (m/z 190, 118, 91) and of MET (m/z 204, 160, 118) were 10.9 and 12.5 min, respectively. LOD was found to be 100 ng/mL.

COC was also extracted using the same LLE described above (without derivatization step) and organic layer was concentrated under N_2 to 30 µL final volume. 1 µL of the sample was used for GC-MS analysis. The column temperature program was as follows: 50 °C (1 min), 50-150 °C (10 °C/min), 150 °C (1 min), 150-280 °C (8 °C/min), 280 °C (15 min). The Rt for COC (m/z 82, 203, 182) was 22.9 min and for methylecgonine (ME; m/z 82, 96, 199) – 12.0 min. LOD was found to be 50 ng/mL.

Identification of OPI in the samples was performed after SPE on Strata Screen C again according to manufacturer's instructions. Dried extract was derivatized using 30 µL EtOAc and 30 µL BSTFA. Mixture was heated at 70 °C for 20 min, and next 1 µL of sample was injected for GC-MS analysis. The column temperature program was as follows: 50 °C (2 min), 50-90 °C (20 °C/min), 90 °C (1 min), 90-280 °C (8 °C/min), 280 °C (10 min). The Rt for BSTFA derivate of morphine (MOR; m/z 439, 236, 146) and of 6-monoacetylmorphine (6-MAM; m/z 399, 400, 340) were 26.5 and 27.2 min, respectively. LOD was found to be 50 ng/mL.

Blood sample (1 mL) underwent a LLE with 4 mL EtOAc in basic media (1 mL 1 M NaOH) after PPT with 2 mL MeCN for GC-MS screening analysis for presence of other drugs. After vortex-mixing and centrifugation (3 000 rpm / 3 min), organic layer was concentrated under N_2 (30 µL final volume) and 1 µL sample was analyzed by GC-MS. The column temperature program was as follows: 50 °C (1 min), 50-150 °C (10 °C/min), 150 °C (1 min), 150-280 °C (8 °C/min), 280 °C (15 min). MS operating conditions were 230 °C ion source temperature, 70 eV, full scan mode (40-550 m/z). Data acquisition and analysis were performed with Agilent MassHunter Workstation software. Drugs were identified using MS data bases NIST, PMWTOX3N, DD2011.

Roadside testing devices Dräger Drug Test 1200 / 5000 cartridges obtained during on-site tests of drivers were opened and test strips were taken off and transferred into test tubes. Thus obtained samples were treated with 3 mL MTBE in presence of 0.1 M NaOH (100 µL, ethanolic solution). After vortex-mixing and centrifugation (3 000 rpm / 3 min), organic layer was concentrated under N_2 (30 µL final volume) and 1 µL sample was analyzed by GC-MS. The column temperature program was same as describe above for screening other drugs of abuse. The Rt for THC (m/z 299, 314, 231), AMP (m/z 44, 91), MET (m/z 58, 91), COC (m/z 82, 303, 182), ME (m/z 82, 96, 199) and heroine (HER; m/z 369, 327, 268) are 28.8, 8.0, 9.0, 22.9, 12.0 and 28.0 min, respectively.

RESULTS AND DISCUSSION

In this study confirmatory drug analysis (blood sample versus roadside OF testing devices) and comparative drug identification study (results from roadside OF testing devices compared with those from their test strips laboratory analysis) was performed. The survey was based on the results obtained by toxicological studies carried out at the Analytical Toxicology Laboratory (Military Medical Academy, Sofia, Bulgaria) in 2013 and 2014. Totally 241 blood samples were submitted by legal authorities and analyzed, among which 67 specimens (28%) were accompanied by complete pre-analytical information regarding drug usage (initial reasons for testing; result from roadside alcohol testing; positive preliminary testing for which drug; when preliminary test was performed). In rest of the cases no detailed preliminary information was available on when, how or in what dosage the drug was taken.

During two-year period surveyed, number of submitted blood samples increased in 2014 (n = 180) as compared to 2013 (n = 61) possibly due to increased enforcement of government regulations and raised efforts by the police. Data showed totally 169 specimens (39 in 2013 and 130 in 2014) in which single or combined illicit drug usage was detected, and 53 (32%) of them were accompanied by full background information.

The age distribution of positively tested persons is presented in Figure 1. The leading age group among users of psychoactive substances is the youngest (18 to 25 years old; 54% in 2013 and 39% in 2014). There are also a significant number of positive results in other groups which confirms the necessity of testing all age groups, not only young drivers.

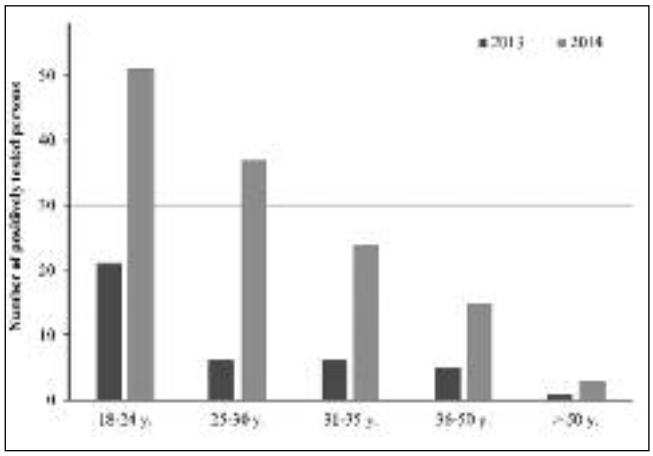

Fig. 1. *Age distribution of positively tested persons*

Type of psychoactive compounds detected during blood testing is shown on Figure 2. The highest results correspond to the single use of THC and to combined usage of illicit drugs. Uptake of other psychoactive substances (benzodiazepines (BZD), methadone (MTD), 3,4-methylenedioxymethamphetamine (MDMA), etc.) by drivers was observed to increase in 2014 as compared to 2013. Some of these detected drugs are regularly applied treating opiate addiction (MTD), epilepsy (valproic acid, carbamazepine), schizophrenia (olanzapine), depression (citalopram, venlafaxine), etc. and cannot be accepted as abused drugs if are prescribed by physicians. In the present study no such preliminary information was submitted by legal authorities.

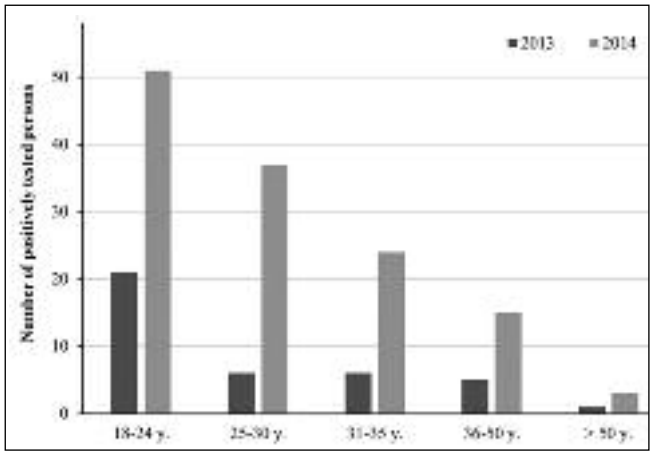

Fig. 2. *Presence of psychoactive drugs in blood specimen of positively tested persons*

Simultaneous use of illicit drugs by positively tested persons include mainly THC and AMP/MET (53% in 2014), but also variety of combinations were observed, based on THC, AMP/MET and OPI (Table 1). Data obtained are in agreement with results published previously for drivers' drug-testing in Bulgaria for 2012 [13].

As it was mentioned above, complete preliminary information about 32% of positive blood samples analyzed in 2013-2014 was available (12 in 2013 and 41 in 2014). From those 53 samples 47 cases of positive preliminary OF on-site test results were confirmed by corresponding blood testing. Comparative analysis of results obtained from laboratory blood tests and on-site OF devices showed a good agreement between both tests (Table 2). The particular cases with mismatching data can be due to false positive result in OF assay based on immune analytical technique.

Table 1. *Number of samples with detected combined usage of illicit drugs*

Combination	2013	2014
THC+AMP/MET	2	28
THC+COC	2	5
THC+OPI	0	3
THC+Other	3	2
THC+AMP/MET+Other	0	2
THC+COC+Other	0	1
THC+OPI+Other	1	1
THC+AMP/MET+COC	0	1
THC+AMP/MET+COC+Other	1	0
AMP/MET+COC	0	1
AMP/MET+Other	1	4
AMP/MET+OPI+Other	0	1
OPI+Other	3	3
Two Other drugs	1	1

Table 2. *Comparison between results from on-site testing device and laboratory blood analysis*

(n = 53 samples, 2013-2014)

Drug(s)	On-site OF testing	Confirmed Blood Analysis	Samples with additional drugs *	Mismatching samples **
THC	24	24	2 (AMP); 2 (COC)	
AMP/MET	12	10	1 (COC); 4 (THC)	1 (OPI); 1 (THC)
COC	1	1	1 (THC)	
OPI	5	5		
THC + AMP	5	4		1 (AMP)
THC + COC	2	1	1 (AMP)	1 (COC)
THC + OPI	1	1		
THC + AMP + COC	1	0		1 (AMP)
BZD	1	1		
THC + BZD	1	0		1 (BZD)

* - presence of additional drugs (in parenthesis) according to laboratory blood analysis

** - disagreement between preliminary OF and laboratory blood results (drug found in blood is presented in parenthesis)

At the next stage we analyzed test strips (as a dried matrix) from 50 used on-site OF immunoassay devices in order to compare results from laboratory instrumental analysis with those obtained from roadside testing. For this purpose roadside testing devices Dräger Drug Test 1200 / 5000 cartridges (Figure 3a, c) were disassembled and their test strips (Figure 3b, d) were taken off and transferred into test tubes. Analysis of test strips from the cartridge device was performed as described above for multi-drug screening. In-lab data of test strips confirmed 50% of preliminary immunoassay cases (Table 3). In other 50% discrepancy was observed and for those cases pre-lab storage period and conditions were unclear (e.g. storage temperature before submitting on-site device to the laboratory). As another important uncertainty factor, the unknown OF sample volume on test strips should be pointed out (unlike Dräger Drug Test 5000, Dräger Drug Test 1200 cartridge does not possess a sample-adequacy indicator).

Table 3. *Comparison of results obtained from preliminary OF immunoassay and in-lab analysis of corresponding test strips*

Drug	Number of on-site OF devices	Number of confirmed results in test strips
THC	24	8
AMP / MET	16	12
COC	5	3
OPI	5	2

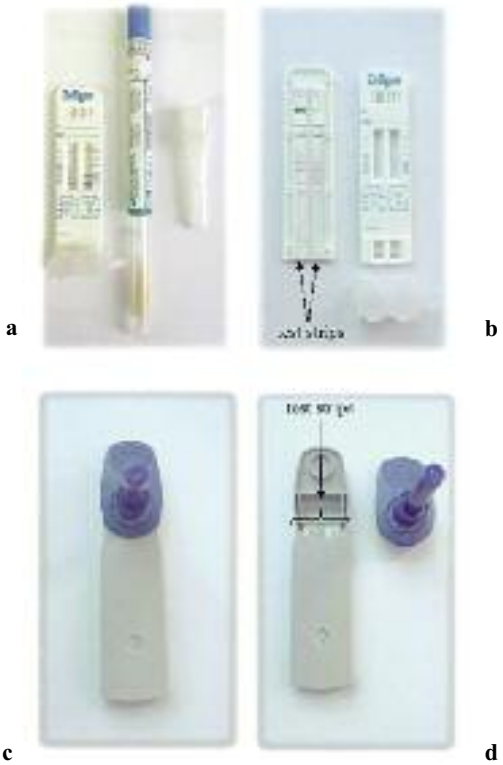

Figure 3. *Roadside testing devices:* **a** *- Dräger Drug Test 1200 cartridge, oral fluid collector and a buffer capsule;* **b** *- opened plastic Dräger Drug Test 1200 cartridge;* **c** *-Dräger Drug Test 5000 cartridge with built-in sample collector;* **d** *- opened ready-to-use Dräger Drug Test 5000 test cassette*

Comparison between on-site and in-lab OF testing results shows that very good correlation was observed for AMP / MET (75%) and for COC (60%), respectively. THC was found to be the main problematic compound for in-lab analysis of test strips from on-site devices (only 33% confirmation). The discrepancy between both tests could be attributed to possible THC adsorption onto the test strip / sample collector and for that reason compound was not sufficiently extracted using selected analytical protocol [14, 15].

CONCLUSION

In the present study we report the results from confirmatory analysis of 241 drivers' blood samples in Bulgaria (2013-2014). Abuse with THC and combined illicit drug use were the most frequently detected cases. Comparison between preliminary roadside OF immunoassay results with those from laboratory blood instrumental analysis shows good correlation between both tests. Comparative study of used on-site OF and in-lab analysis of their test strips (as dried matrix) shows that latest could be used for toxicological testing when no other sample (blood / urine) is submitted for analysis. The best result was found for AMP/MET and COC. The survey revealed that it is necessary to continue monitoring of both matrices (OF and blood) for the purposes of DUID control and forensic toxicology.

Sažetak

Potvrdna analiza uzoraka krvi na prisustvo sredstava zloupotrebe sprovedena je u Bugarskoj u periodu 2013-2104. Ispitivanje je izvršeno na 241 uzorku vozača za koje se sumnjalo da su vozili pod dejstvom psihoaktivnih supstanci. Dobijeni rezultati upoređenisu sa rezultatima dobijenim nakon preliminarnog testiranja pljuvačke. Istraživanje je pokazalo da je najveći broj ljudi koji su vozili pod uticajem psihoaktivnih supstanci starosne dobi između 18 i 25 godina. Podaci dobijeni nakon analize krvi govore o tome da je najčešće zloupotrebljavana supstanca tetrahidrokanabinol. Takođe, česti su slučajevi kombinovanja više zabranjenih psihoaktivnih supstanci. Dodatna komparativna analitička studija 50 uzoraka analiziranih pomoću uređaja za testiranje na licu mesta i test traka pokazala je da laboratorijska analiza test traka kao osušenog matriksa može biti zadovoljavajuća alternativa testovima iz krvi. Rezultati pokazuju da je potrebna kontinuiranaanaliza oba matriksa (pljuvačke i krvi) za potrebe praćenja vozača koji zloupotrebljavaju psihoaktivne supstance i sudske toksikologije.

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