Drugs of abuse testing in dry oral fluid spot

S. STOYKOVA[1,2], V. ATANASOV[1,2,*]

[1]Laboratory of Biocoordination and Bioanalytical Chemistry, Faculty of Chemistry and Pharmacy, Sofia University "St. Kl. Ohridski", Sofia, Bulgaria;
[2]Analytical Toxicology Laboratory, Emergency Toxicology Clinic, Military Medical Academy, Sofia, Bulgaria

- **Introduction:** The oral fluid (OF) became a widely used sample for drugs of abuse preliminary screening, particularly in on-site (roadside) testing. However, confirmatory analysis must be performed in most cases when the initial test is based on immunoassay. The dry OF spot as an analogue to dry blood spot can be applied as a suitable technique for sampling, storage and transportation of the specimen avoiding effect of time, temperature and microorganism growing as well as fast sample-preparation procedure.

- **Methods:** Dry OF spots were prepared from devices used in roadside OF tests (Dräger Drug Test 1200/5000 cartridges, Fig. 1) or from collected OF specimens. Sample (100-200 µL) was placed on 3-layers filter paper (Fig. 2) and dried on air at room temperature. The obtained dry OF spot (Fig. 3) underwent methyl *tert*-butyl ether (MTBE) extraction (3 mL) in the presence of 100 µL 0.1 M NaOH in ethanol. After sonication (3 min) the extract was separated and concentrated under nitrogen up to 50 µL and analyzed by gas chromatography-mass spectrometry (GC-MSD; Agilent 7890B/5977A Series). The GC is fitted with a HP-5ms capillary column (30 m x 0.25 mm x 0.25 µm). The procedure for sample preparation of the dry OF spots was optimized by testing acetonitrile and MTBE as extragents, and using 0.1 M NaOH or 1 M ammonia in ethanol on spots containing spiked OF with amphetamine (AMP, 0.4 µg), methadone (MTD, 0.2 µg), cocaine (COC, 0.2 µg) and morphine (MOR, 0.4 µg). To compare analytical results between samples an internal standard – 100 µg/mL lidocaine (50 µL) was used only in the model samples. A comparative study of 71 cases, preliminary tested using Dräger Drug Test 1200/5000 STK was performed.

Fig. 1: Dräger Drug Test 1200/5000 cartridges.

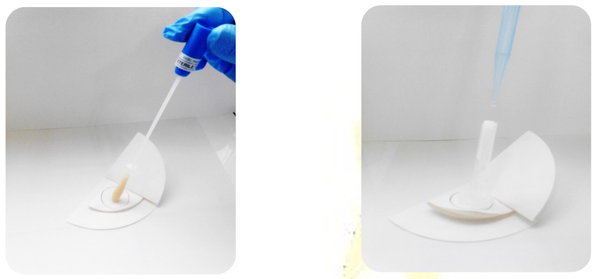

Fig. 2: Preparation of dry OF spot using Dräger Drug Test 1200/5000 STK.

Fig. 3: A - Dry OF spot; B – Sample prep dry OF spot .

- **Results:** The described dry spots of spiked OF have a net mass of 7-10 mg (200 µL, single drop technique). The results obtained revealed that the suitable sample preparation of dry OF spot is MTBE extraction in presence of 0.1 M NaOH in ethanol. Representative chromatogram is shown in Fig. 4.

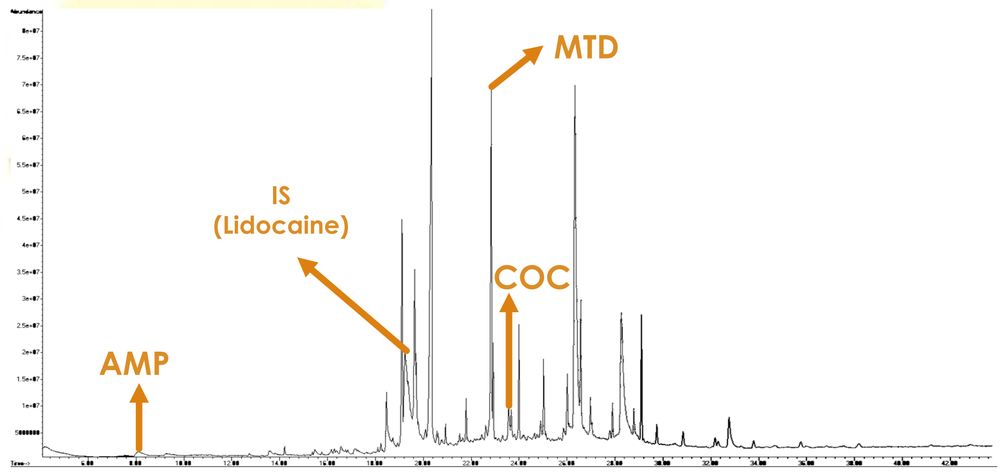

Fig. 4: A chromatogram of spiked dry OF spot, developed by the suitable sample preparation.

Using the procedure described above it is possible to detect and confirmed the presence of different psychoactive drugs as MTD, codeine, heroine, acetylcodeine, COC, AMP, metamphetamine in OF. The presence of each compound found in dry OF spot was verified by liquid-liquid extraction of the native OF (Fig. 5). Comparison on both chromatograms shows more purified extract from dry spot and different recovery (34% for AMP, 74% - MTD and 145% - COC of LLE extraction).

From the comparative study of real samples admitted in the laboratory for confirmatory analysis in blood the highest number of discrepancies was found for AMP, metamphetamine and MDMA – 5/11 were false positive, which implies that chromatographic analysis of OF will be good alternative.

At the same time an increased number of combined drug usage was detected, which was named "THC-based" (13/38) and "MTD-based" (5/38). However, in all cases instrumental confirmatory analyses is needed and dry spot technique is a suitable alternative to blood test or when continuing storage of the samples in necessary.

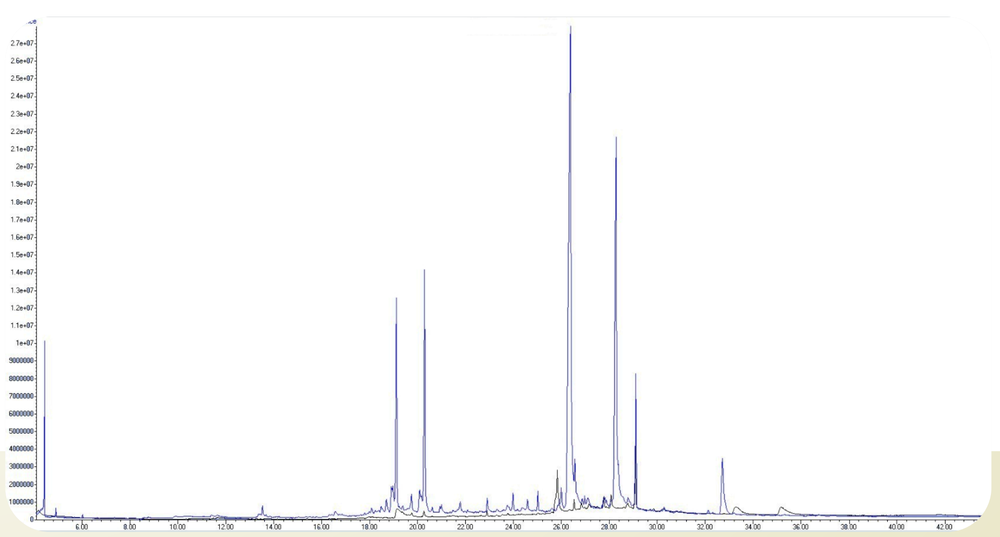

Fig. 5: A chromatogram of dry OF spot (blue) and of native OF (black) from the same person. In both samples codeine, acetylcodeine and heroine are identified.

GC-MS condirtions:
The oven temperature is held initially at 50°C for 1 min, programmed from 50 to 150°C at 10°C/min, and held at 150°C for 1 min and from 150 to 280°C at 8°C/min, and held at 280°C for 15 min. The electron ionization MS operating conditions is as follows: 230°C ion source temperature and 70 eV electron energy. The MSD is operated using SIM and full scan mode with range of 40-550 m/z.

- **Conclusion:** The analytical procedure presents a direct analysis of dry OF samples. The advantages of spot analysis are stable sample, on-"spot" pre-concentration (several loads on single spot), fast and simple sample pretreatment with decreased matrix contamination. The described technique is a good alternative for laboratory confirmatory analyses on used roadside devices, when positive result is indicated.

* e-mail: vatanasov@chem.uni-sofia.bg

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Acknowledgments: This document has been prepared with the financial assistance of the European Social Fund (Grant BG051PO001-3.3.06-0040 and Grant BG051PO001-3.3.06-0048.

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This document has been prepared with the financial assistance of the European Social Fund. Sofia University "St. Kliment Ohridski"– Faculty of Medicine bears full responsibility for the content of this document and in no circumstances can be regarded as official position of the European Union or the Bulgarian Ministry of Education and Science.