Widespread illegal use of psychoactive substances is the reason for the development of fast, sensitive, accurate and non-invasive analytical methods to identify the narcotics used by drivers/criminals on-site, so that appropriate preventive measures can be taken. For such a method to be useful, several factors have to be considered, including non-invasive sampling and sample preparation time, the portability of instruments, instrument start-up time, and actual analysis time.

To date, the preliminary assessment of illicit cannabis use employs on-site screening tests of oral fluid. Their main drawbacks are cross-reactivity and poor analyte recovery from the device, which leads to low diagnostic sensitivity (about 60%).

Present study aims to demonstrate a potential of capillary electrophoresis (CE) method, its exceptional power of separation and resolution, short analysis time and ability for miniaturization for on-site assessment of illegal drugs use. In this project we translated the proof of the principle, demonstrated on a home-made lab scale instrument onto a truly portable (30 x 30 x 18 cm) instrument for on-site measurements.

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Different in-house built portable minituarized CE instruments were developed in Tallinn University of Technology for different environmental, medical, and forensic applications.

Developed CE system (highlighted in red box) with UVC excitation source (λex 230 nm) allowed native fluorescence detection of twelve different illegal drugs such as amphetamines, cocaine and its derivative, and cannabinoids at their cut-off limits.

The use of oral fluid (OF) for drug abuse testing is easy, sampling is non-invasive and does not require any special training. In this study we succeeded to combine saliva sample collection/preparation/pre-concentration procedure into one step using the Salivette sampling device (shown in PROCEDURE schematics). No separate precipitation of proteins and/or derivatisation was required. The whole procedure including saliva sample collection, clean-up and CE separation of analytes, took less than 20 minutes.

It was also possible to couple the developed CE system with different detectors such as conductivity detector. This allowed to analyze also GHB, psilocybin and ibotenic acid abuse in saliva samples.
Developed methodologies were thoroughly validated according to EMA Guidelines. Linearity of calibration curve, limits of detection and quantification (LOD and LOQ), selectivity against endogenous and exogenous interferences, inter-day precision, accuracy, carry-over effects, extraction recovery and matrix effect were evaluated.

The developed CE-FD system allowed to differentiate drug impairment levels taking into account DRUID recommended detection capability levels and the latest publications by H. Gjerde, where the drug impairment levels were studied, relying on the illegal drugs concentration levels in blood versus oral fluid.
Besides testing CE apparatus in the lab environment with real saliva samples provided by police, the portable instrument was also used for on-site analysis on the Baltic Weekend Festival held in Estonia in August 2016. Saliva samples were collected during the law enforcement officers ride.

40 positive samples were determined by CE-FD: 17 samples containing amphetamine, 23 samples containing MDMA, 15 samples containing cocaine, 5 samples containing THC.

The majority of saliva samples contained different drugs’ mixtures. The following illegal drugs mixtures were found: MDMA+amphetamine (7 samples), cocaine+MDMA (5 samples), amphetamine+cocaine+MDMA (5 samples), cocaine + alcohol (cocaethylene as a marker) (10 samples), amphetamine + cocaine (1 sample), MDMA + MDA (1 sample).

Moreover, CE-FD fingerprints were analyzed by hierarchical cluster analysis. Ten different main clusters were defined. Several of them are shown in this slide. The CE-FD fingerprints allowed to find possible linkages between donors and can be potentially used by police for case investigation.
CASE STUDY: ANALYSIS OF ORAL FLUID SAMPLES AFTER THE CONTROLLED AD LIBITUM CANNABIS CIGARETTE SMOKING AND COLLECTED REAL SAMPLES

In this study we succeeded to determine the cannabis abuse. The real saliva sample of frequent user was collected with the Salivette® device after the controlled ad libitum smoking of one cannabis cigarette (20 mg THC and 15 mg CBD) during one hour from the smoking start. The new detection system was capable to determine even the trace levels of THC and CBD in the blank sample after previous use. The CE-FD system was capable to differentiate the illegal cannabis use from legal cannabis products consumption, where immunoassays can fail, giving the false positive result for products, containing CBD.

The exceptional power of separation and resolution, short analysis time, economical use of reagents, and minimum sample requirements make CE an attractive methodology for forensic laboratories. The developed sensitive FD coupled to CE is a promising tool for direct determination of illegal drug abuse on the road within the minutes’ timeframe with minimum sample pre-treatment and no separate precipitation of proteins or derivatisation.