Detection and determination of methadone in oral fluid from patients admitted to a drug-maintenance program

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Introduction: Methadone (MTD, Fig. 1) is a synthetic narcotic acting as an analgetic and a sedative. Its main therapeutic usage is in methadone maintenance programs.
Oral fluid (OF) is an alternative matrix that may have advantages over blood and urine for drug analysis among patients in treatment programs. It is a complex liquid secret (composition similar to serum) which can be collected painlessly, rapidly, non-invasively and the risk of adulteration is considered to be much lower. The **aim** of the present study is to investigate if OF can be used as alternative to urine and blood samples to monitor drug abuse in opioid-dependent patients in MTD maintenance therapy.

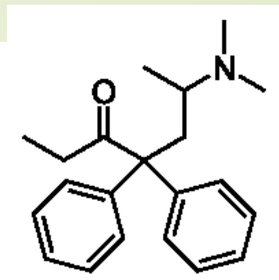

Fig. 1: Chemical structure of methadone (MTD).

Methods: OF specimen (unstimulated) is obtained from opioid-dependent volunteers receiving daily 50 -150 mg MTD. Urine and blood samples are taken at the same time. OF samples underwent acetonitrile precipitation, ethylacetate liquid-liquid extraction and MTD is identified using gas chromatography-mass spectrometry (GC-MSD; Trace GC/DSQ, Thermo). The amount of MTD was measured using liquid chromatography (HPLC-UV). The analysis of MTD in urine is performed after liquid-liquid extraction (NaOH, ethylacetate). The quantitative analysis of MTD in blood samples is performed by solid-phase extraction on mixed mode sorbent (Strata Screen C).
The GC analysis is carried on BP-35 capillary column (30 m x 0.25 mm x 0.25 µm). The MSD is operated in scan mode (40-550 m/z). HPLC analysis (Shimadzu LC 20 A pump / SpectraSystem 2000 Uv-Vis detector, Thermo) has been used for quantitative determination of MTD. Analysis is performed on reversed-phase C18 stationary phase column (1.0 mL/min flow rate) followed by UV detection at 240 nm. The mobile phase is consisted of 65% 10 mM triethylamine-phosphate buffer pH 2.7 and 35% acetonitrile.
The creatinine (CR) content in all OF samples is measured using Jaffé reaction.

Results: There is correspondence between OF and urine screening for MTD and its primary metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). The data shown a good correlation in identified drugs by the screening performed as well as MTD presence. Representative chromatograms of the OF and urine screening of the same patient are presented in Fig. 2.

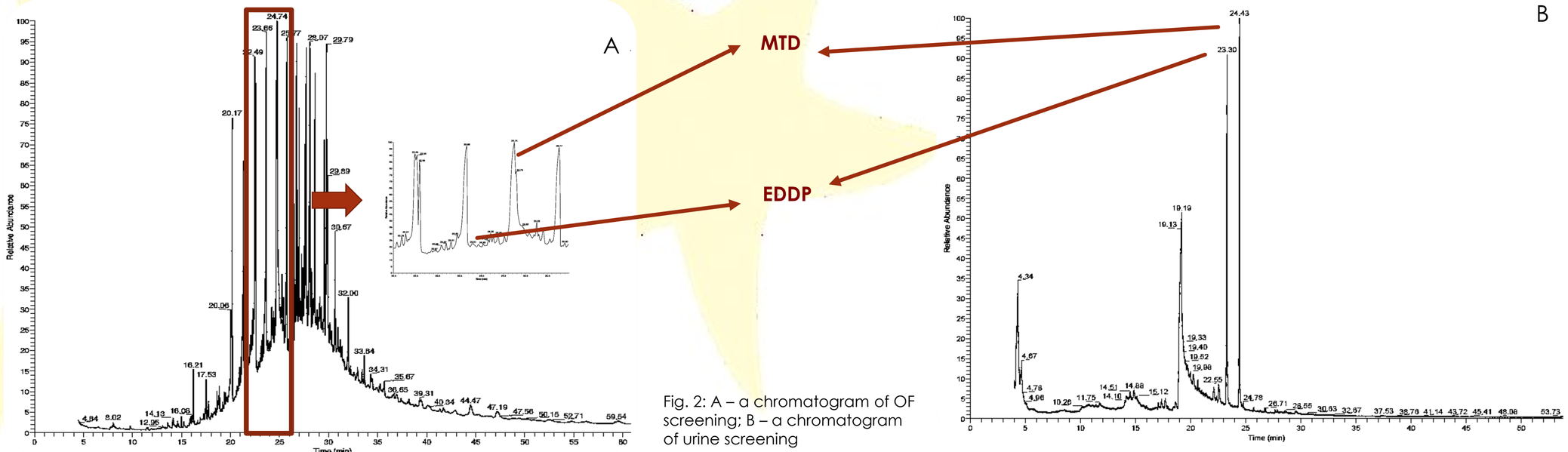

Fig. 2: A – a chromatogram of OF screening; B – a chromatogram of urine screening

Representative data from quantitative determination of MTD in OF and blood is shown in Fig. 3. MTD in OF was measured in concentration range from 0.08 to 3.10 µg/mL depending on the initial dose and time of sampling. The result is not directly related to MTD blood concentration, as the kinetic is different in both samples. However, correlation between blood and OF is better when CR-normalization is applied.

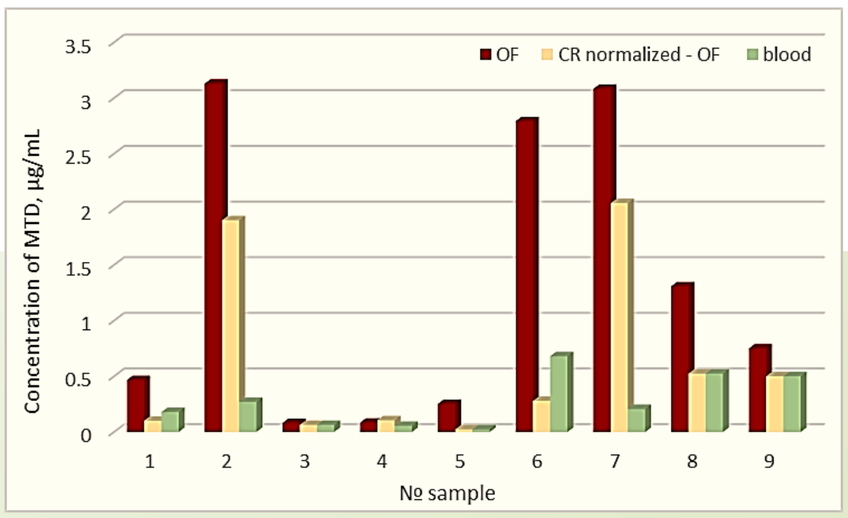

Fig. 3: Graphical representation of quantitative analysis of MTD in selected samples of OF and blood.

Conclusion: OF can be considered as a suitable non-invasive sample for screening of MTD in opioid-dependent patients subjective on substitutive therapy. Depending on time of specimen collection after MTD dosing, the concentration of MTD is highly variable in OF sample while that in blood seems to be more stable in time.
Normalization to creatinine content of OF improves the correlation with MTD blood concentration.

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