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New automated technique for the simultaneous detection of some addictive drugs in saliva samples with the Fast GC/MS SPME Multifiber System

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AIMS: The aim of the work is to assess the suitability of different methods for the sensitive identification, screening and quantitation of some addictive drugs of abuse in saliva by comparing eight selected analytical procedures. The Authors present a method that employs sequential mixed mode by new automated SPME Multifiber System, and optimized derivative formation for short-column Fast GC/MS. With SPME Multifiber System, the multi-fiber analysis sequence works in a simple way: the fibers are transported between the 25-position tray, the vials and the injector by the new holder equipped with a plunger/magnetic system. At the end of the analysis the desorbed fiber is moved back to the tray and the cycle is repeated. Moreover, the autosampler parameters used for all derivatisation reagents (by sample tray rotation or magnetic stirrer) allowed to complete the analysis cycle by conditioning and sealing in the Teflon septum inserted into the tray.

METHODS: Methadone, 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium perchlorate (EDDP), cocaethylene, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethyl amphetamine (MDEA), N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB), cannabidiol (CBD), D9-tetrahydrocannabinol (THC), cannabinol (CBN) were analyzed by head space (HS)/direct immersion (DI)-SPME while amphetamine, methamphetamine and cocaine were analyzed either with in situ derivatization (hexyl-, methyl-, propyl- and butylchloroformate) or without derivatization. Headspace SPME was performed for ethanol. Benzodiazepines (oxazepam, diazepam, nordiazepam, flunitrazepam) were analyzed either by conversion to the corresponding benzophenones or with solvent modified SPME. The following fiber coatings were used: 100, 30 and 7 µm polydimethylsiloxane (PDMS), 65 µm Carbowax/Divinylbenzene (CW/DVB), 85 µm polyacrilate (PA), 65 µm DVB/PDMS (Sigma-Aldrich Srl, Supelco).
GC-MS analyses were performed on a Shimadzu GC 2010 with the system acquisition GC Solution software using an SLB5-MS™ column (5 m × 0.10 mm × 0.4 µm film thickness) from Sigma-Aldrich Srl, Supelco with a Shimadzu QP 2010 series mass selective detector operating in the EI mode (Shimadzu Italia Srl, Italy). Automation of the procedure was achieved using a CombiPal autosampler from CTC Analytics (Alfatech SpA) equipped with SPME Multifiber system from ChromLine (Alfatech SpA).

RESULTS: All the methods were shown to be highly reproducible and robust with respect to variations in the saliva matrices. However, in Fast GC/MS separation, temperature ramping and high carrier gas flow-rate combined with short column reduced the analysis time and achieved sharp peak shapes while still maintaining sufficient resolution and high sample capacity. Validated parameters including selectivity, linearity, accuracy, intra- and inter-day precision (coefficient of variation < 10%), extraction efficiency and limit of quantitation (methadone, EDDP, MDMA, MDEA; MBDB, CBD, THC, CBN 1-100 ng/ml by HS-SPME with 30 µm PDMS fiber, amphetamine, methamphetamine and cocaine 0.5-5 ng/ml by in situ derivatization with butylchloroformate and 100 µm PDMS fiber; ethanol 15 mg/l by HS/SPME with 100 µm PDMS fiber; benzodiazepines 500-2000 ng/ml with solvent modified SPME by 85 µm polyacrylate fiber).

CONCLUSIONS: Starting by the theoretical considerations which determine the passive ab/adsorbtion of the analytes in a sampling SPME system, the Authors of the present work have developed a system able to analyse great quantities of samples in a very short time, by reducing this way the costs of the monitoring campaigns. The aim is to extend the use of this system to all working facilities, such as public and private laboratories, which require rapid, reliable determinations. Fully automated SPME procedure could to be more readily acceptable for such purposes as toxicological screening, clinical diagnosis, etc.

REFERENCES:

Fucci N, De Giovanni N, Chiarotti M., Simultaneous detection of some drugs of abuse in saliva samples by SPME technique, Forensic Sci Int. 2003: 24; 134(1): 40-5.

Mills GA, Walker V., Headspace solid-phase microextraction procedures for gas chromatographic analysis of biological fluids and materials, J Chromatogr A. 2000: 1; 902(1): 267-87.

Application of Solid Phase Micro Extraction. Edited by Janusz Pawliszyn. RSC Chromatography Monographs. Series Editor: Roger M. Smith, University of Technology, Loughborough, UK 1999.

Reubsaet KJ, Ragnar Norli H, Hemmersbach P, Rasmussen KE. Determination of benzodiazepines in human urine and plasma with solvent modified solid phase micro extraction and gas chromatography; rationalization of method development using experimental design strategies. J Pharm Biomed Appl 1998: 18 (4-5): 667-680.

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