K7 A Method for the Determination of Amphetamines and Methylenedioxyamphetamines in Oral Fluid by Gas Chromatography/ Mass Spectrometry

Aimee M. Halphen, MS, and C. Richard Crooks, PhD, Kroll Laboratory Specialists, Inc., 450 Southlake Boulevard, Richmond, VA 23236; Michelle*
R. Peace, PhD, Virginia Commonwealth University, Kroll Laboratory Specialists, Inc., 1000 West Franklin Street, Richmond, VA 23284; and Alphonse Poklis, PhD, Medical College of Virginia, Box 98-165, Virginia Commonwealth University/Medical College of Virginia Hospital Station, Richmond, VA 23298

Upon reviewing this poster presentation, observers will become familiar with a validated GC/MS method for detecting and quantifying amphetamines and methylenedioxyamphetamines in oral fluid, which may be easily applied in a forensic drug testing laboratory. This presentation will impact the forensic science community by demonstrating how the increase in popular- ity of using oral fluid in forensic drug testing has provoked a need for improved and reliable methods for drug extraction, detection, and quantita- tion. SAMHSA is currently evaluating oral fluid (OF) in an attempt to provide a set of universal standards for laboratories. This study yields data which may be applicable to developing future SAMHSA guidelines.

The authors present the validation of a gas chromatography/mass spectrometry (GC/MS) method for the detection and quantification of amphetamine (AMP), methamphetamine (MAMP), 3,4-methylene-dioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyehtylamphetamine (MDEA) in oral fluid. Prior to extraction, 500 uL of oral fluid was pretreated with 250 uL of 0.7M sodium periodate for 15 min. The sample was then made basic with 200 uL 1N potassium hydroxide and extracted with 1.0 mL of n-butyl chloride. After separation, the extracted amphetamines were derivatized with heptaflurobu- tyic anhydride (HFBA) including removal of excess HFBA by washing with 1N potassium hydroxide and water. The amphetamines were separated and quantified in an Agilent 5973 GC/MS equipped with a HP-Ultra 1, 12m X 0.2mm X 0.33um capillary column with a 4mm splitless liner. The oven temperature program was: initial 60°C for 0.2 min., then ramped at 20°C/min. to 180°C, held for 0 min., then ramped at 2°C to 185°, held for 0 min. Under these conditions the retention times in minutes of amphetamine HFBA derivatives were: AMP, 4.75; MAMP, 5.40; MDA, 6.68; MDMA, 7.54; MDEA, 7.95. The drugs were quantified with their respective deuter- ated species as internal standards. The MSD was operated in the SIM mode monitoring the following m/z ions: AMP-HFB, 240, 91 and 118; MAMP- HFB, 254, 210 and 118; MDA-HFB, 162, 240 and 375; MDMA-HFB, 254, 210 and 389; MDEA-HFB, 268, 240 and 403; $^{2}H_{10}$-AMP-HFB, 244 and 97; $^{2}H_{11}$-MAMP-HFB, 260 and 213; $^{2}H_{5}$-MDA-HFB, 167 and 380; and $^{2}H_{5}$- MDMA-HFB, 258 and 213; and $^{2}H_{16}$-MDEA-HFB, 274 and 244. Amphetamine and MDA displayed a linear range 10-2000 ng/mL with
a 10 ng/mL LOQ and LOD. Methamphetamine was found linear from 5-2000 ng/mL with a 5 ng/mL LOQ and LOD. The assay was less sensitive for MDMA and MDEA with a LOQ and LOD of 20 ng/mL; however, the assay was linear up to 3750 ng/mL for these analytes. The method yielded excellent precision. At a proposed cut value of 50 ng/mL and + 25% of this cut-off (target values 37.5 ng/mL and 62.5 ng/mL), the %CV values were <5% for each amphetamine at the three target concentrations. The method was applied to specimens obtained with two different oral fluid collection devices; the Intercept (Orasure Technologies) and the Salivette (Sarstedt). A notable interference with the AMP-HFB 91 m/z ion and the MDEA-HFB 240 m/z ion was observed in oral fluid collected with the Intercept device. No interferences were observed with specimens collected by the Salivette device. The present method was found to be reliable for the determination of amphetamine, methamphetamine and their commonly abused methyl- enedioxy-derivatives in oral fluid.

Oral Fluids, Amphetamines, MDMA

 ** Presenting Author*