

# Method development and validation for analysis of amphetamine, methamphetamine, methylenedioxy derivatives and metabolites in sweat patches using disk solid-phase extraction and gas chromatography-mass spectrometry

BRUNO S. DE MARTINIS<sup>1</sup>, ALLAN J. BARNES<sup>2</sup>,  
KARL B. SCHEIDWEILER<sup>2</sup> and MARILYN A. HUESTIS<sup>2</sup>

<sup>1</sup> Department of Pathology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Center of Legal Medicine, Ribeirão Preto, SP, 14051-140, Brazil

<sup>2</sup> Chemistry and Drug Metabolism, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, 5500 Nathan Shock Dr., Baltimore, MD 21224, USA.

The recreational use of amphetamines and their derivative 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") has spread worldwide due to their stimulant and hallucinogenic potential. We describe a sensitive and specific method for simultaneous quantification of amphetamine (AMP), methamphetamine (MAMP), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxyethylamphetamine (MDEA), 4-hydroxy-3-methoxyamphetamine (HMA) and 4-hydroxy-3-methoxymethamphetamine (HMMA) from sweat patches using disk solid phase extraction (SPE) and gas chromatography-mass spectrometry (GC/MS).

Deuterated analogs were utilized as internal standards for all analytes except HMMA and HMA where a closely related deuterated analyte was employed. Blank sweat patches were moistened with artificial sweat, spiked with working calibrator and internal standard solutions, and extracted on a reciprocating shaker using 3 mL of 0.2 M sodium acetate buffer (pH 5.0). A 1 mL aliquot of the buffered extract was applied to SPE columns (SPEC MP1, Varian Inc.) preconditioned with methanol, water and 0.1 M acetic acid. Columns were washed with water, 0.1 M acetic acid, and methanol and eluted with ethyl acetate: methanol: ammonium hydroxide (78:20:2). Methanolic hydrochloric acid was added to the eluate prior to evaporation under nitrogen. Extracts were reconstituted with 100  $\mu$ L 0.1 M triethylamine in heptane and derivatized with 10  $\mu$ L heptafluorobutyric acid anhydride (HFAA) at 60°C. After cooling, 0.05 M Tris buffer (pH 7.4) was added, samples vortexed, centrifuged and the organic layer was transferred to vials for analysis by electron impact GC/MS in the SIM mode.

In each analytical run, two calibration curves with a weighting factor of 1/x were constructed for each analyte in order to establish extended calibration ranges. Low calibration curves were constructed from 2.5 – 500 ng/patch for MAMP, AMP, MDMA and MDEA, 5 – 500 ng/patch for MDA, and 5 – 100 ng/patch for HMA and HMMA. High calibration curves at 500 – 10000 ng/patch were constructed for MAMP, AMP, MDA, MDMA and MDEA, and a smaller dynamic range of 100 – 2500 ng/patch was utilized for HMA and HMMA. Coefficients of determination (R<sup>2</sup>) for calibration curves (N=6) were  $\geq$  0.990. The limits of detection (LOD) and quantification (LOQ) for AMP, MAMP, MDMA and MDEA were 2.5 ng/patch, and 5 ng/patch for MDA, HMA and HMMA. Within-run (N=4) and between-run (N=24) precision was calculated at six control levels (7.5, 75, 300, 750, 3000 and 6000 ng/patch) with coefficients of variation of less than 7.5%. Accuracies at the same concentrations were +/- 9.5% of target concentrations.

Similarly, mean extraction efficiencies for all controls (N=4) were in the range of 84.7% - 112.1% for all analytes. Forty potential interfering compounds were individually spiked (5000 ng/patch) into low quality control samples (7.5 ng/patch) to evaluate specificity. All controls quantitated within +/- 20% of target and showed no interferences with analytes or internal standards. This method will be employed to investigate the distribution of MDMA and metabolites into human sweat following controlled administration of MDMA.

**KEYWORDS:** *3,4-Methylenedioxymethamphetamine (MDMA), Metabolites, Sweat, GC/MS*

**Corresponding author:** [martinis@usp.br](mailto:martinis@usp.br)