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Development and validation of a disk solid phase extraction and gas chromatography–mass spectrometry method for MDMA, MDA, HMMA, HMA, MDEA, methamphetamine and amphetamine in sweat

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Abstract

We describe the development and validation of a method for the simultaneous quantification of 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3-hydroxy-4-methoxymethamphetamine (HMMA), 3-hydroxy-4-methoxyamphetamine (HMA), 3,4-methylenedioxyethylamphetamine (MDEA), methamphetamine (MAMP) and amphetamine (AMP) in sweat. Drugs were eluted from PharmChek™ sweat patches with sodium acetate buffer, extracted with disk solid phase extraction and analyzed using GC/MS-EI with selected ion monitoring. Limits of quantification (LOQ) for MDMA, MDEA, MAMP and AMP were 2.5 ng/patch, and 5 ng/patch for MDA, HMA and HMMA. This fully validated procedure was more sensitive than previously published analytical methods and permitted the simultaneous analysis of multiple amphetamine analogs in human sweat.
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Keywords: MDMA; Amphetamine; Methamphetamine; Sweat patches; GC/MS

1. Introduction

Detection of drugs in sweat was proposed by the Substance Abuse Mental Health Services Administration's (SAMHSA) Mandatory Guidelines for Federal Workplace Drug Testing Program in April 2004 [1]. Sweat has been investigated as an alternate biological matrix for monitoring drug use [2–8]. Sweat patches are a convenient and tamper-evident means of collecting sweat. A sweat patch consists of a rectangular, absorbent, cellulose pad attached to an adhesive polyurethane backing. Prior to attaching the patch, skin is cleaned with an alcohol wipe to remove external contamination from drug in the environment and to improve adherence. According to Kidwell and Smith [9], inappropriate cleansing of the skin prior to patch placement can result in contamination of the patch. In addition, these investigators believe that the cellulose pad can become contaminated by drug in the environment, although the experimental designs used to demonstrate this phenomenon were uncharacteristic of common exposure conditions. Sweat testing has several advantages over blood and urine including non-invasive collection, reduced opportunity for sample adulteration, and in some cases, longer detection windows than plasma or urine. Disadvantages of sweat as an alternative specimen include lack of information about dose–response relationships, lower analyte concentrations and a shortage of laboratories performing sweat analysis. Detection of 3,4-methylenedioxymethamphetamine (MDMA) and metabolites is important in forensic toxicology for workplace drug testing, criminal justice, drug abuse treatment and sport doping control programs. Although sympathomimetic amphetamines have been reported in human perspiration [10], little is known about MDMA and metabolite disposition in sweat [5,7,8]. Proposed SAMHSA requirements for a positive sweat test include an amphetamines screen $\geq$25 ng/patch, and a confirmation cutoff of 25 ng/patch for MDMA, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxyethylamphetamine (MDEA), methamphetamine (MAMP) and/or amphetamine (AMP). For MAMP confirmation, the specimen must also contain AMP at

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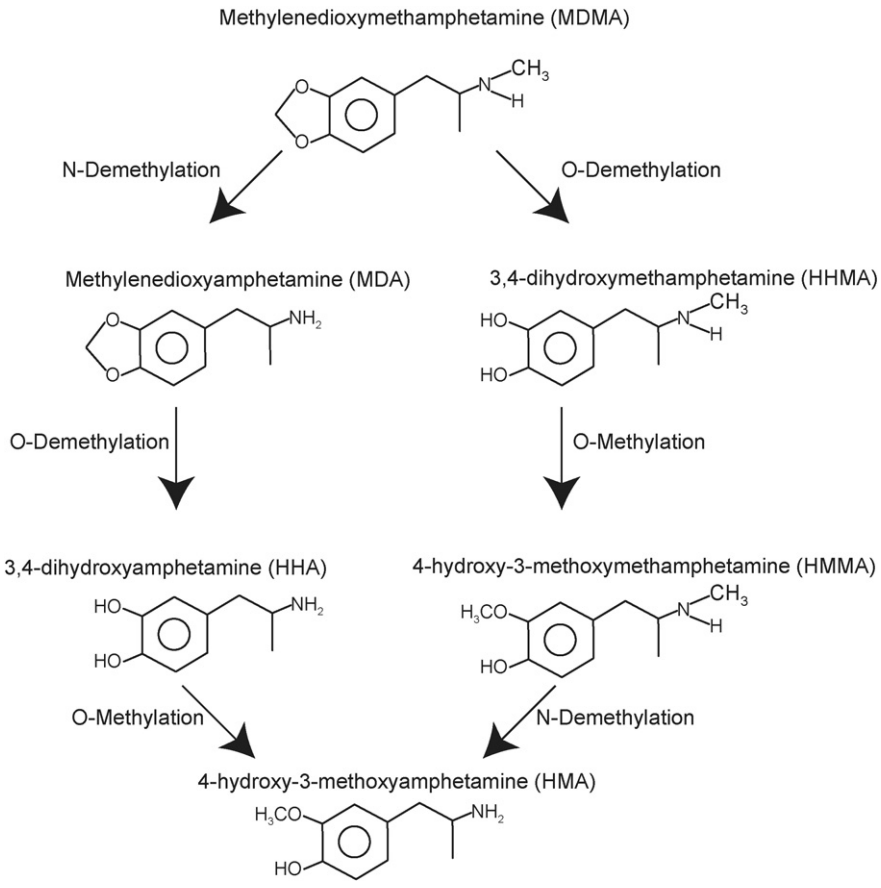

Fig. 1. Metabolic pathway for methylenedioxymethamphetamine (MDMA).

a concentration greater than or equal to the method's limit of detection (LOD).

MDMA, or "ecstasy", is a commonly abused synthetic psychoactive substance. Consumption of MDMA and other sympathomimetic amines including MDA, MDEA, MAMP and AMP is widespread and increasingly popular among young people in the United States and Europe [11–13]. MDMA is a stimulant, usually taken in oral tablet form, resulting in hallucinogenic effects approximately 20 min after drug intake and lasting from 4 to 6 h [14,15]. Psychological effects include euphoria, increased confidence, sensuousness, enhanced empathy and facilitation of contact. Adverse effects are described as dry mouth, tachycardia, confusion, depression, paranoia, muscle tension, involuntary teeth clenching, nausea, blurred vision, tremors and sweating [14,16]. MDMA is frequently associated with dance parties known as "raves", where the risk of dehydration, hyperthermia and hyperpyrexia is enhanced, due to excessive physical activity and increased body temperature [17]. Controversy over MDMA's long-term toxicity centers on its effects on the dopaminergic and serotonergic systems [18,19].

MDMA metabolism is complex and includes two main metabolic pathways. *N*-Demethylation of MDMA produces MDA. MDMA and MDA are *O*-demethylated to 3,4-dihydroxymethamphetamine (HHMA) and 3,4-dihydroxyamphetamine (HHA), respectively. HHMA and HHA are intermediate metabolites that are subsequently *O*-methylated to 4-hydroxy-3-methoxymethamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA) (Fig. 1). HMMA and HMA are primarily excreted in the urine as conjugated glucuronide or sulphate metabolites [20,21]. There are few data on the disposition of these metabolites in alternative matrices.

Sweat is a slightly acidic matrix, with a pH ranging from 4 to 6.8. Amphetamine-related compounds are weak bases with low protein binding facilitating the transfer of lipid-soluble compounds from blood to other fluids and/or tissues. This can lead to an accumulation in biological samples with pH values lower than that of plasma (pH 7.4) or ion trapping of the drugs in the more acidic matrix [8,16,22,23]. The other important factor in the transfer of drug into sweat is the lipophilicity of the compound. Generally, lipophilic (less polar) compounds have a greater ability to cross biological membranes. Sweat wipes [24,25] and sweat patches [5,8,26] have been analyzed for sympathomimetic amines. Although some methods simultaneously quantified MDMA, MDA, MDEA, MAMP and AMP [5,24,25] in sweat; no method to date has included HMMA and HMA.

This study describes the development and validation of an analytical method for the simultaneous quantification of amphetamine, methamphetamine, methylenedioxy derivatives and metabolites in human sweat using disk solid phase extraction (SPE) and gas chromatography–mass spectrometry (GC/MS). This is the first report to fully describe method validation parameters including MDMA metabolites, HMMA and HMA, in sweat collected with the PharmChek^{TM} sweat patch. The inclusion of

these compounds in our analytical method was undertaken to evaluate if these analytes are useful biomarkers of MDMA consumption and if their identification and quantification provide unique information for the interpretation of MDMA sweat test results.

2. Experimental

2.1. Chemicals and reagents

Ampules of AMP, MAMP, MDMA, MDEA, MDA, HMMA (1 mg/mL in methanol) and the internal standards AMP-d_{11}, MAMP-d_{14}, MDMA-d_5, MDA-d_5 and MDEA-d_6 (100 μg/mL in methanol) were purchased from Cerilliant Corporation (Round Rock, TX, USA). HMA was obtained from Lipomed Inc. (Cambridge, MA, USA). In addition, potential interferents: pseudoephedrine, norpseudoephedrine, phenylpropanolamine, phentermine, diphenhydramine, *p*-methoxymethamphetamine, *p*-methoxyamphetamine, clonidine, fenfluramine, ibuprofen, acetylsalicylic acid, brompheniramine, chlorpheniramine, nicotine, caffeine, pentazocine, phencyclidine, methadone, 6-acetylmorphine, morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, Δ^9-tetrahydrocannabinol, 11-hydroxy-Δ^9-tetrahydro-cannabinol, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol, cocaine, benzoylecgonine, norcocaethylene, norcocaine, *m*-hydroxycocaine, *p*-hydroxycocaine, *m*-hydroxybenzoylecgonine, *p*-hydroxybenzoylecgonine, ecgonine ethyl ester, ecgonine methyl ester, anhydroecgonine methyl ester and gamma hydroxy butyrate also were purchased from Cerilliant Corporation.

Heptafluorobutyric acid anhydride (HFAA) was obtained from Pierce Chemical Co. (Rockford, IL, USA). SPEC MP1 disk solid phase extraction cartridges (10 mL/70 mg) were from Varian Inc. (Lake Forest, CA, USA). Tris [hydroxymethyl] aminomethane base, Tris [hydroxymethyl] aminomethane hydrochloride, triethylamine (99.5% purity), ammonium chloride, lactic acid and heptane (GC grade) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Concentrated hydrochloric acid, acetic acid, ammonium hydroxide, potassium phosphate monobasic, potassium phosphate dibasic, sodium chloride, sodium hydroxide, urea, methanol (HPLC grade) and ethyl acetate (HPLC grade) were purchased from JT Baker (Phillipsburg, NJ, USA). PharmChek™ sweat patches were supplied by PharmChem Inc. (Fort Worth, TX, USA). Artificial sweat solution contained 327 mmol/L ammonium chloride, 166 mmol/L lactic acid, 83 mmol/L urea, 42 mmol/L acetic acid, 34 mmol/L sodium chloride in deionized water and pH was adjusted to 4.7 with 2 mol/L sodium hydroxide.

2.2. Calibrators and quality controls

Purchased stock solutions (1 mg/mL) of MAMP, AMP, HMA, MDA, HMMA, MDMA and MDEA were combined and diluted with methanol to yield working calibrator solutions (0.1, 1.0, 10.0 and 100 μg/mL). Working calibrator solutions were added to blank sweat patches to create daily calibration curves from 2.5 to 10,000 ng/patch. Quality control (QC) solutions, at the same concentrations, were prepared in methanol from different lots than used for calibrator solutions. Solutions of deuterated MAMP, AMP, MDA, MDMA and MDEA were diluted in methanol to produce a working internal standard solution containing all analogs at 1 μg/mL. Deuterated analogs were used as internal standards for most compounds, with the exception of HMA and HMMA. In the absence of commercially available stable isotopes for these analytes, MDA-d_5 and MDMA-d_5 were employed as internal standards for HMA and HMMA, respectively. All solutions were stored in amber glass vials at −20 °C. Unused drug-free sweat patches were moistened with 750 μL of artificial sweat evenly distributed onto the pad of each patch and allowed to dry for 2 h at room temperature. For preparation of calibration curves, these patches were spiked with standard solutions yielding concentrations of 2.5, 5, 10, 25, 50, 100, 250, 500, 1000, 2500, 5000, 7500 and 10,000 ng/patch. Internal standards were added to each patch at concentrations of 25 ng/patch. QC samples also were prepared daily by spiking these blank patches with control solutions to yield concentrations of 7.5, 75, 300, 750, 3000 and 6000 ng/patch for all analytes and 25 ng/patch of deuterated internal standard. After fortification, all patches were allowed to air dry for 30 min at room temperature.

2.3. Extraction and derivatization procedure

Patches containing spiked calibrators, QC samples or clinical specimens were folded (twice) and placed into 17 mm × 60 mm screw top vials. Three millilitres of 0.2 M sodium acetate buffer (pH 5.0) was added to each vial, prior to securing on a horizontal reciprocating shaker (250 RPM) for 30 min. A portion of the buffered extract (1 mL) was applied to SPEC MP1 columns preconditioned with 1 mL of methanol, 1 mL of distilled water and 500 μL of 0.1 M acetic acid. SPE columns were washed sequentially with 500 μL of distilled water, 250 μL of 0.1 M acetic acid, 400 μL of methanol and dried for 1 min with 30 psi of compressed air, using a System 48-positive pressure manifold (SPEware Corporation, San Pedro, CA, USA). Analytes of interest were eluted into clean 5 mL centrifuge tubes using two 1 mL aliquots of freshly prepared elution solvent; ethyl acetate:methanol:ammonium hydroxide (78:20:2, v/v/v). Fifteen microlitres of 1% hydrochloric acid in methanol (v/v) was added to each eluate before vortexing and evaporating under nitrogen at 35 °C. Dried extracts were reconstituted using 100 μL of 0.05 M triethylamine in heptane and 10 μL of heptafluorobutyric acid anhydride (HFAA). Centrifuge tubes were capped, vortexed and incubated for 20 min at 60 °C. After cooling to room temperature, 200 μL of 0.05 M Tris buffer, pH 7.4 was added and tubes vortexed for 2 min in a multi-tube vortex mixer. Samples were centrifuged at room temperature, 2200 × *g* for 5 min and organic layers transferred to autosampler vials for analysis by GC/MS.

2.4. Chromatographic and detection system conditions

The identification and quantification of derivatized extracts was performed using an Agilent 6890 gas chromatograph

Table 1
Analytes, internal standards, and target and qualifier ions for the analysis of methylenedioxy derivatives and metabolites and methamphetamine and amphetamine in human sweat

Analyte	Retention time (min)	Target ion (*m/z*)	Qualifier ion(s) (*m/z*)
Amphetamine-d_{11} (AMP-d_{11})	3.36	244	98
Amphetamine (AMP)	3.39	240	118, 91
Methamphetamine-d_{14} (MAMP-d_{14})	4.03	261	213
Methamphetamine (MAMP)	4.09	254	210, 118
4-Hydroxy-3-methoxy-amphetamine (HMA)[a]	5.56	240	360, 163
3,4-Methylenedioxyamphetamine-d_5 (MDA-d_5)	5.97	167	380
3,4-Methylenedioxyamphetamine (MDA)	6.00	162	375, 135
4-Hydroxy-3-methoxy-methamphetamine (HMMA)[b]	6.30	254	360, 210
3,4-Methylenedioxymethamphetamine-d_5 (MDMA-d_5)	6.91	258	213
3,4-Methylenedioxymethamphetamine (MDMA)	6.93	254	162, 210
3,4-Methylenedioxyethylamphetamine-d_6 (MDEA-d_6)	7.00	274	244
3,4-Methylenedioxyethylamphetamine (MDEA)	7.02	268	240, 162

[a] MDA-d_5 utilized as internal standard.
[b] MDMA-d_5 utilized as internal standard.

coupled with an Agilent 5973 quadrupole mass selective detector operated in electron impact mode (Agilent Technologies, Wilmington, DE, USA). The temperatures of the quadrupole, ion source and mass selective detector interface were 150, 230 and 280 °C, respectively. Derivatized extracts (1 μL) were injected using a split ratio of 1:5. The injection port temperature was maintained at 170 °C. The initial oven temperature was held at 70 °C for 1 min followed by ramps of 25 °C/min to 130 °C, 10 °C/min to 160 °C and 40 °C/min to a final temperature of 300 °C. Chromatographic separation was achieved within 9.9 min using a DB-35 ms bonded-phase capillary column (15 m × 0.32 mm i.d. × 0.25 μm film thickness) and high purity helium (99.999%) was used as carrier gas at a flow rate of 2.5 mL/min. Mass selective detection was achieved by operating in the selected ion monitoring mode with the electron multiplier set to 200 V relative to the daily tuning parameter. Three ions for each analyte and two ions for each internal standard were monitored. A list of retention times and monitored ions for each analyte are presented in Table 1.

2.5. Data analysis

Compounds were identified by comparing retention times (±2%) and qualifier ion ratios (±20%) to the corresponding average values of calibrators assayed in the same batch. Peak abundance ratios of analytes to the corresponding internal standards were calculated for each concentration. Calibration was performed with Agilent MSD Chemstation software (Version D.00.00). Data were fit to a linear least-squares regression curve with a weighting factor of 1/*x*. In addition, calibrator concentrations, when calculated against the full calibration curve, were required to be within (±20%) of the target value.

In each analytical batch, two calibration curves were constructed for each analyte in order to achieve adequate sensitivity and linearity. Low calibration curves were constructed from 2.5 to 500 ng/patch for MDMA, MDEA, MAMP and AMP, 5–500 ng/patch for MDA and 5–100 ng/patch for HMMA and HMA. High curves 500–10,000 ng/patch were constructed for MDMA, MDA, MDEA, MAMP and AMP, and a smaller dynamic range of 100–2500 ng/patch was utilized for HMMA and HMA.

2.6. Method validation and acceptance criteria

The method was validated by determining specificity, linearity, sensitivity, carry-over, accuracy, precision (inter- and intra-assay), extraction efficiency and stability.

2.6.1. Specificity

Specificity was defined as the ability to identify and quantify analyte with or without the presence of endogenous or exogenous components. Blank sweat patches ($N=6$) were worn by drug-free volunteers ($N=6$) to verify the absence of potential endogenous interferents or adverse matrix effects. In addition, this method was challenged with 40 potentially interfering substances including structurally similar or commonly co-administered compounds, metabolites and over-the-counter medications. Low QC sweat patches (7.5 ng/patch) were spiked individually to contain 5000 ng/patch of pseudoephedrine, norpseudoephedrine, phenylpropanolamine, phentermine, diphenhydramine, *p*-methoxymethamphetamine, *p*-methoxyamphetamine, clonidine, fenfluramine, ibuprofen, acetylsalicylic acid, brompheniramine, chlorpheniramine, nicotine, caffeine, pentazocine, phencyclidine, methadone, 6-acetylmorphine, morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, Δ^9-tetrahydrocannabinol, 11-hydroxy-Δ^9-tetrahydrocannabinol, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol, cocaine, benzoylecgonine, norcocaethylene, norcocaine, *m*-hydroxycocaine, *p*-hydroxycocaine, *m*-hydroxybenzoylecgonine, *p*-hydroxybenzoylecgonine, ecgonine ethyl ester, ecgonine methyl ester, anhydroecgonine methyl ester and gamma hydroxybutyrate.

Each analyte of interest was required to be adequately resolved from other analytes and tested interferents, have acceptable chromatographic parameters and quantitative analyte concentrations within 20% of expected.

2.6.2. Linearity and sensitivity

Calibration curves for linearity determination were prepared daily by analyzing unused sweat patches pre-moistened with artificial sweat, and spiked to contain 2.5, 5, 10, 25, 50, 100, 250, 500, 1000, 2500, 5000, 7500 and 10,000 ng/patch for analytes of interest. Linearity was determined by the method of least squares and expressed as the coefficient of determination (R^2). A $1/x$ weighting factor was utilized. Calibrators were required to satisfy all identification criteria and quantify within 20% of target concentration.

Sensitivity was evaluated by determining the limits of detection (LOD) and quantification (LOQ) of the assay. The LOD was evaluated in triplicate and defined as the lowest concentration for which the signal-to-noise ratio for all ions (determined by peak height) was at least 3, and chromatography exhibited acceptable peak shape, retention time (±2% of target) and qualifier ion ratios (within ±20% of the average ion ratios of all calibrators). The LOQ was established as the lowest concentration that met all LOD criteria and had analyte concentrations within ±20% of target.

2.6.3. Precision and accuracy

Precision and accuracy were evaluated over the linear dynamic range using six QC samples at target concentrations of 7.5, 75, 300, 750, 3000 and 6000 ng/patch. Intra-assay data for each analyte were collected within one analytical run ($N=4$), and inter-assay data were determined from four replicates in six separate runs ($N=24$). Data were evaluated using one-way analysis of variance with day as the grouping variable. Precision was expressed as percent relative standard deviation and calculated by dividing the mean analyte concentration by the standard deviation and multiplying by 100. Accuracy, expressed as a percentage, was determined by taking the difference of mean calculated and target concentrations.

2.6.4. Extraction efficiency

Extraction efficiency for each analyte was determined at 7.5, 75, 300, 750, 3000 and 6000 ng/patch. Internal standard and working calibrator solutions were spiked into one set ($N=4$) of blank patches prior to extraction. Internal standard was added to another set ($N=4$) of patches prior to extraction and working calibrator solution was added to the eluates after SPE prior to evaporation. Samples were derivatized and analyzed. Extraction efficiency was calculated by comparing mean concentration of each analyte in the set spiked prior to SPE to the corresponding mean concentration in the set spiked after SPE.

2.6.5. Stability

Stability of analytes may be influenced by storage and handling conditions. Stability of spiked unextracted sweat patches was tested in triplicate at all QC concentrations after undergoing three freeze–thaw cycles, after refrigeration at 4 °C for 72 h and after 12 h at room temperature. Concentrations of control stability samples were compared to freshly prepared and analyzed calibration curves. Stability of derivatized samples also was evaluated. Autosampler vials were stored at room temperature and re-injected up to 48 h after initial analysis. Stability criteria included acceptable ion ratios (±20% average of calibrator ratios) and quantification of QC samples within 20% of expected concentrations.

2.7. Clinical study

To verify that the method was adequate for monitoring MDMA and metabolites in sweat, the presence of MDMA, MDA, HMMA and HMA were determined in sweat after controlled administration of MDMA to a healthy individual with a history of Ecstasy use. The subject provided informed consent to participate in this National Institute on Drug Abuse (NIDA) Intramural Research Program Institutional Review Board (IRB) approved study. A sweat patch was applied for various periods prior to, during and after MDMA administration. A 1.0 mg/kg of MDMA was administered orally to a participant who resided on the secure clinical unit throughout the study.

3. Results

To evaluate specificity, sweat patches were worn for a minimum of 12 h by drug-free volunteers ($N=6$). The skin was thoroughly cleaned with an alcohol pad (70% isopropyl alcohol) and patches were applied to the chest. Patches were extracted and analyzed to assess potential interferences from endogenous substances and matrix effects. No interferences were detected. In addition, in each analytical run ($N=6$), a blank and negative sample (blank sweat patch with internal standard) were evaluated. Also, 40 potential drug interferents, including structurally similar compounds, metabolites, co-administered drugs and over-the-counter medications were individually spiked (5000 ng/patch) into low (7.5 ng/patch) QC samples. All QC sample results were within 20% of target concentration and fulfilled chromatographic and ion ratio criteria for all analytes of interest.

In each analytical run, two calibration curves with a weighting factor of $1/x$ were constructed for each analyte in order to extend the dynamic range of the assay. Low calibration curves were constructed from 2.5 to 500 ng/patch for MDMA, MDEA, MAMP and AMP, 5–500 ng/patch for MDA and 5–100 ng/patch for HMMA and HMA. High calibration curves at 500–10,000 ng/patch were constructed for MDMA, MDA, MDEA, MAMP and AMP, and a smaller dynamic range of 100–2500 ng/patch was utilized for HMMA and HMA. Calibrators were required to have acceptable chromatography, ion ratios and quantify with ±20% of target concentrations. Coefficients of determination (R^2) for all calibration curves ($N=6$) were ≥ 0.990 for all analytes (Table 2). LOD and LOQ for MDMA, MDEA, MAMP and AMP were 2.5 ng/patch, and 5 ng/patch for MDA, HMMA and HMA (Table 2). Representative chromatography illustrating extracted ion chromatograms of sweat patches fortified with drug at each analytes' LOQ are presented in Fig. 2. Patches ($N=3$) exceeding the linear range for all analytes at 20,000 ng/patch were extracted and analyzed to evaluate carryover. Immediately following each carryover sample, a negative sample containing only internal standard was injected, allowing

Table 2
Limits of detection and quantification, and calibration curves ($N = 6$) for methylenedioxy derivatives and metabolites and methamphetamine and amphetamine in human sweat

Compound	LOD[a] (ng/patch)	Linear range (ng/patch)	Regression equation $y = m^b x + b^c$	Coefficients of determination (R^2)
Amphetamine	2.5	2.5–500	$y = 0.041(0.001)x + 0.017(0.040)$	0.997–0.999
		500–10,000	$y = 0.045(0.002)x - 2.680(0.917)$	0.997–0.999
Methamphetamine	2.5	2.5–500	$y = 0.042(0.001)x - 0.016(0.007)$	0.993–0.999
		500–10,000	$y = 0.045(0.001)x - 1.473(1.751)$	0.998–1.000
HMA[d]	5	5–100	$y = 0.078(0.004)x - 0.035(0.045)$	0.993–0.999
		100–2500	$y = 0.070(0.003)x - 9.066(2.421)$	0.996–0.998
MDA[e]	5	5–500	$y = 0.170(0.004)x - 0.083(0.071)$	0.996–0.999
		500–10,000	$y = 0.191(0.011)x - 1.856(3.089)$	0.993–0.999
HMMA[f]	5	5–100	$y = 0.043(0.002)x - 0.021(0.011)$	0.997–0.999
		100–2500	$y = 0.050(0.002)x - 3.200(0.968)$	0.998–0.999
MDMA[g]	2.5	2.5–500	$y = 0.075(0.004)x - 0.046(0.022)$	0.990–0.999
		500–10,000	$y = 0.108(0.009)x - 4.327(2.493)$	0.994–0.999
MDEA[h]	2.5	2.5–500	$y = 0.046(0.001)x - 0.015(0.007)$	0.997–0.999
		500–10,000	$y = 0.050(0.002)x - 1.295(0.587)$	0.998–0.999

[a] Limit of detection.
[b] Slope (S.D.).
[c] Intercept (S.D.).
[d] 4-Hydroxy-3-methoxy-amphetamine.
[e] 3,4-Methylenedioxyamphetamine.
[f] 4-Hydroxy-3-methoxy-methamphetamine.
[g] 3,4-Methylenedioxymethamphetamine.
[h] 3,4-Methylenedioxyethylamphetamine.

us to quantify potential carryover from the previous injection. There was no evidence of carryover at the method's LOD.

Precision and accuracy of the method were evaluated at three concentrations over the linear range of each curve for MDMA, MDA, MDEA, MAMP and AMP and at two concentrations across each HMMA and HMA calibration curve. Data for intra-assay ($N = 4$) in each of six assays and inter-assay ($N = 24$) precision and accuracy are presented in Tables 3 and 4. Inter-assay precision (%R.S.D.) ranged from 1.3% to 7.4%, while intra-assay precision was less than 5.8% on six different days. Inter-assay accuracy ($N = 4$, assays = 6), calculated as the percent difference between mean and target concentrations was between 95.4% and 110.8%. Control concentrations for each analyte were evaluated using a single-factor analysis of variance with day as the grouping variable. The data showed statistically significant differences between days for all analytes ($p = 0.05$); however,

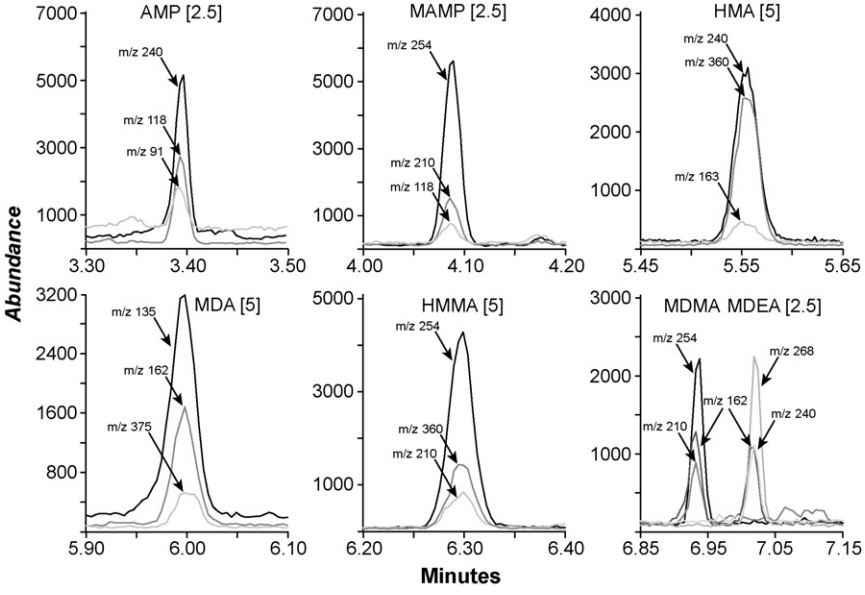

Fig. 2. Merged extracted ion chromatograms from a sweat patch spiked at the limit of quantification for all analytes.

Table 3
Precision (% R.S.D.) data for methylenedioxy derivatives and metabolites and methamphetamine and amphetamine in human sweat

Compound	Inter-assay ($N=24$) (ng/patch)						Intra-assay ($N=4$) (ng/patch)					
	7.5	75	300	750	3000	6000	7.5	75	300	750	3000	6000
Amphetamine	7.4	4.2	3.5	1.5	1.6	1.4	4.3	3.3	3.7	0.8	3.1	1.1
Methamphetamine	4.4	4.8	3.2	4.0	1.4	1.3	4.8	2.9	4.5	0.4	3.2	0.6
HMA[a]	5.9	6.9	4.4	5.4	[f]	[f]	5.4	1.0	2.3	5.7	[f]	[f]
MDA[b]	3.9	6.4	4.8	3.4	3.0	1.6	3.8	3.1	3.7	2.1	4.0	1.0
HMMA[c]	5.8	5.6	7.2	5.6	[f]	[f]	4.3	4.6	3.1	2.9	[f]	[f]
MDMA[d]	4.5	5.2	4.7	2.6	1.9	1.6	2.2	3.3	3.2	1.7	3.6	1.3
MDEA[e]	5.0	4.8	4.4	2.8	1.9	1.4	3.9	4.1	3.5	0.5	3.4	1.1

[a] 4-Hydroxy-3-methoxy-amphetamine.
[b] 3,4-Methylenedioxyamphetamine.
[c] 4-Hydroxy-3-methoxy-methamphetamine.
[d] 3,4-Methylenedioxymethamphetamine.
[e] 3,4-Methylenedioxyethylamphetamine.
[f] Upper limit of linearity = 2500 ng/patch.

differences in daily mean analyte concentrations did not exceed 12.5% of target and were considered clinically insignificant.

Extraction efficiencies were calculated by comparing mean concentrations ($N=4$) of QC samples prepared by adding analyte before and after SPE. The method provided mean percent extraction efficiencies of 84.7–112.1% (Table 5).

Stability studies were conducted to ensure that analytes were stable during collection, processing and preparation. Patches ($N=3$) were spiked at six concentrations (7.5, 75, 300, 750, 3000, 6000 ng/patch) and placed in a −20 °C freezer for 16 h, removed and left at ambient temperature for 8 h, and the process repeated two additional times. Mean analyte concentrations of these stability samples were compared to target concentrations with results from 88.3% to 108.9%. Analyte concentrations, evaluated in triplicate, for short-term (12 h room temperature) and long-term (4 °C for 72 h) stability experiments were within ±14.5% of target for all analytes. Stability of derivatized extracts in capped GC autosampler vials at room temperature was assessed after 24 and 48 h. Derivatized control samples ($N=3$) were stable, with acceptable quantification within 16.5% of target for all compounds of interest up to 48 h after initial injection.

4. Discussion

There are a variety of testing matrices available to monitor illicit drug use. Testing for drugs of abuse in unconventional specimens such as sweat has become possible with the development of highly sensitive instrumentation. The greatest challenges to the analysis of drugs in sweat are reproducible analyte recovery from the sweat patch and the need for high sensitivity. We present a fully validated GC/MS method for the simultaneous analysis of MDMA, MDA, HMMA, HMA, MDEA, MAMP and AMP in sweat. To the best of our knowledge, the present study appears to be the first to fully describe method validation parameters for the simultaneous quantification of these analytes.

Pichini et al. [8] measured the methylenedioxy derivatives MDMA, MDA and HMMA in sweat patches following a single oral administration of 100 mg of MDMA, and reported LOQ's for MDMA and MDA of 10 ng/patch and HMMA of 2.5 ng/patch. Kintz et al. [5] applied sweat patches to 20 volunteers in a detoxification center and reported linear ranges of 10–500 ng/patch for MDMA, MDA, MDEA, MAMP and AMP.

Table 4
Inter-assay accuracy data (% of target concentration) for GC/MS quantification of methylenedioxy derivatives and metabolites and methamphetamine and amphetamine in human sweat ($N=24$)

	Target (ng/patch)					
	7.5	75	300	750	3000	6000
Accuracy (% of target concentration)						
Amphetamine	99.7	99.7	105.3	102.7	99.4	99.7
Methamphetamine	102.8	97.8	103.6	100.5	101.6	101.7
HMA[a]	104.7	105.9	110.8	107.6	[f]	[f]
MDA[b]	101.4	95.4	103.2	99.3	109.5	103.0
HMMA[c]	100.2	106.0	101.5	100.7	[f]	[f]
MDMA[d]	101.5	96.5	103.2	100.1	99.4	98.5
MDEA[e]	101.5	97.0	103.2	100.3	102.2	100.1

[a] 4-Hydroxy-3-methoxy-amphetamine.
[b] 3,4-Methylenedioxyamphetamine.
[c] 4-Hydroxy-3-methoxy-methamphetamine.
[d] 3,4-Methylenedioxymethamphetamine.
[e] 3,4-Methylenedioxyethylamphetamine.
[f] Upper limit of linearity = 2500 ng/patch.

Table 5
Mean extraction efficiency (%) of methylenedioxy derivatives and metabolites and methamphetamine and amphetamine from sweat patches ($N = 4$)

Curve	QC concentration (ng/patch)	2.5–500 AMP[a]	2.5–500 MAMP[b]	5–100 HMA[c]	5–500 MDA[d]	5–100 HMMA[f]	2.5–500 MDMA[e]	2.5–500 MDEA[g]
Low	7.5	97	102.5	89.4	105.4	99	104.8	101.5
Low	75	96.6	94.9	85.5	94.1	84.7	94.1	93.8
Low	300	97.7	93.8	[h]	92.7	[h]	89.6	89.4
Curve	QC concentration (ng/patch)	500–10,000 AMP[a]	500–10,000 MAMP[b]	100–2500 HMA[c]	500–10,000 MDA[d]	100–2500 HMMA[f]	500–10,000 MDMA[e]	500–10,000 MDEA[g]
High	300	[i]	[i]	102.9	[i]	97.5	[i]	[i]
High	750	104.1	100.1	112.1	90.5	98.2	99.5	96.3
High	3000	99.9	100.5	[h]	109	[h]	100.2	99.9
High	6000	106.9	111.3	[h]	105.7	[h]	105.4	111.8

[a] Amphetamine.
[b] Methamphetamine.
[c] 4-hydroxy-3-methoxy-amphetamine.
[d] 3,4-Methylenedioxyamphetamine.
[e] 4-Hydroxy-3-methoxy-methamphetamine.
[f] 3,4-Methylenedioxymethamphetamine.
[g] 3,4-Methylenedioxyethylamphetamine.
[h] Above analyte linearity.
[i] Below analyte limit of quantification.

With the exception of HMMA (5 ng/patch), the current assay lowers quantification limits by a factor of 2–4, without the need for expensive hyphenated analytical techniques. This enhanced sensitivity will increase the windows of drug detection for these analytes, and should enable a more thorough investigation of the disposition of amphetamine analogs and metabolites in sweat. In addition, the proposed SAMHSA guidelines require a confirmation cutoff of ≥25 ng/patch, which is easily achieved with the present method.

The upper limit of linearity is 2500 ng/patch for HMA and HMMA and up to 10,000 ng/patch for all other analytes. These extended dynamic ranges allow quantification of seven amphetamine analogs and metabolites with a single extraction and injection, reducing both laboratory and analysis time. Extraction efficiencies (>84.7%) at six concentrations across the linear dynamic range were consistent with published data. The method also achieved acceptable within and between-run precision and accuracy (≤10.8%).

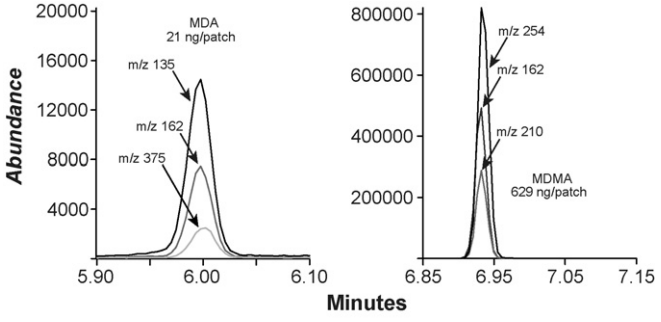

Fig. 3. Merged extracted ion chromatograms from a sweat patch collected 5 h after administration of 1.0 mg/kg of MDMA to a volunteer with a history of Ecstasy use. The patch contained 629.0 ng/patch MDMA and 21.0 ng/patch MDA.

Matrix effects and interferences from non-targeted compounds are important considerations in forensic applications. Our specificity experiment documented that concentrations (5000 ng/patch) of 40 commonly used licit and illicit drugs did not interfere with accurate quantification of the low QC (7.5 ng/patch) for all analytes. In addition, measured analyte concentrations in short and long-term stability studies were within ±20% of target.

This validated analytical method was applied to the measurement of MDMA and metabolites in sweat collected from a participant in a controlled oral MDMA administration study. Fig. 3 shows representative chromatography from a sweat patch applied 1.25 h prior to administration of 1.0 mg/kg of MDMA. The patch was removed 5 h after administration and was found to contain 629 ng/patch MDMA and 21 ng/patch MDA. HMMA and HMA were not identified at the LOD.

This sensitive and specific method permits simultaneous and accurate quantification of methylenedioxy derivatives and metabolites and MAMP and AMP in human sweat and should be useful for workplace drug testing, criminal justice and drug abuse treatment programs. This method will be applied to sweat specimens collected from participants enrolled in controlled MDMA administration studies.

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