Published in final edited form as:
Anal Bioanal Chem. 2011 April ; 400(1): 69–78. doi:10.1007/s00216-010-4392-2.

Simultaneous analysis of buprenorphine, methadone, cocaine, opiates and nicotine metabolites in sweat by liquid chromatography tandem mass spectrometry

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Abstract

A liquid chromatography tandem mass spectrometry method for buprenorphine (BUP), norbuprenorphine (NBUP), methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), cocaine, benzoylecgonine, ecgonine methyl ester (EME), morphine, codeine, 6-acetylmorphine, heroin, 6-acetylcodeine, cotinine, and trans-3′-hydroxycotinine quantification in sweat was developed and comprehensively validated. Sweat patches were mixed with 6 mL acetate buffer at pH 4.5, and supernatant extracted with Strata-XC-cartridges. Reverse-phase separation was achieved with a gradient mobile phase of 0.1% formic acid and acetonitrile in 15 min. Quantification was achieved by multiple reaction monitoring of two transitions per compound. The assay was a linear 1–1,000 ng/patch, except EME 5–1,000 ng/patch. Intra-, inter-day and total imprecision were <10.1%CV, analytical recovery 87.2–107.7%, extraction efficiency 35.3– 160.9%, and process efficiency 25.5–91.7%. Ion suppression was detected for EME (−63.3%) and EDDP (−60.4%), and enhancement for NBUP (42.6%). Deuterated internal standards compensated for these effects. No carryover was detected, and all analytes were stable for 24 h at 22 °C, 72 h at 4 °C, and after three freeze/thaw cycles. The method was applied to weekly sweat patches from an opioid-dependent BUP-maintained pregnant woman; 75.0% of sweat patches were positive for BUP, 93.8% for cocaine, 37.5% for opiates, 6.3% for methadone

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and all for tobacco biomarkers. This method permits a fast and simultaneous quantification of 14 drugs and metabolites in sweat patches, with good selectivity and sensitivity.

Keywords

Buprenorphine; Methadone; Cocaine; Cotinine; Sweat; Drug monitoring

Introduction

Alternative matrices in forensic toxicology, sweat, oral fluid and hair, are receiving increasing interest due to ease and non-invasiveness of collection and/or longer windows of drug detection. Sweat offers several advantages compared to blood and urine. Sweat is collected with patches that are generally worn for 1 week, permitting drug monitoring over a longer period of time than urine with a single sweat patch. Wearing the patch is frequently considered less invasive and embarrassing, and awareness of the patch may help deter relapse to drug use and alter behavior [1]. Sweat patches are mainly used in treatment, workplace, and criminal justice monitoring programs, but on-site fast drug detection in sweat also is possible [2, 3].

Sweat composition is approximately 99% water with the most concentrated solute being sodium chloride [4]. Passive diffusion of drugs from capillaries in the skin into perspiration is the primary pathway of drug incorporation in sweat, but excretion of substances via sebum and intercellular diffusion also contribute [5]. Excretion into sweat depends upon a drug's physicochemical properties such as molecular mass, pK_a, protein binding, and lipophilicity. Parent drugs are generally excreted at higher concentrations than metabolites [6] due to their greater lipophilicity, and basic compounds tend to accumulate in sweat due to ion-trapping in the more acidic conditions [3].

Sweat patches resemble a large Band-Aid® that is applied to the back, upper arm, or lower chest and is generally worn for about 7 days. The non-occlusive, semi-permeable membrane allows oxygen, carbon dioxide, and water vapor to escape. The adsorbent pad retains the nonvolatile components of sweat, such as salts, proteins, and drugs and metabolites [7]. In general, sweat patches adhere well and produce little irritation [3, 8].

The Substance Abuse Mental Health Service's Administration's (SAMHSA) Mandatory Guidelines for Federal Workplace Drug Testing [9] proposed sweat as a useful alternative matrix in follow-up drug testing and treatment programs. Although not yet finalized, the guidelines proposed initial and confirmatory test cutoff concentrations (ng/patch) of 1 for THC, 25 for cocaine, opiates, and amphetamines, and 20 for phencyclidine.

A recent review describes the few articles on analysis of drugs in sweat [10]. Several methods were published for the determination of opiates [11, 12], cocaine [13, 14], buprenorphine (BUP) [15], methadone [16], and nicotine [17] in sweat. Skopp et al. [18] adapted a hair methadone method for sweat, and Cone et al. [6] a blood and oral fluid heroin, cocaine and metabolites method for sweat. Only two published gas chromatography mass spectrometry (GCMS) methods permit simultaneous quantification of different classes of drugs in sweat, such as cocaine, opiates and methadone [7], and cocaine, opiates, THC and benzodiazepines [15].

We developed a liquid chromatography tandem mass spectrometry (LCMSMS) sweat method to monitor BUP compliance, determine tobacco-smoking status, identify relapse to opiates and cocaine, and use of diverted methadone in opioid-dependent pregnant women

receiving BUP pharmacotherapy. To our knowledge, this is the first sensitive and selective LCMSMS method to simultaneously quantify 14 drugs and metabolites in a single sweat patch.

Materials and methods

Chemicals and materials

Morphine, codeine, 6-acetylmorphine (6AM), heroin, 6-acetylcodeine (6AC), cocaine, benzoylecgonine (BE), ecgonine methyl ester (EME), cotinine, BUP, norbuprenorphine (NBUP), methadone and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) standards, and morphine-d_6, 6AM-d_6, heroin-d_9, cocaine-d_3, BE-d_8, EME-d_3, cotinine-d_3, BUP-d_4, NBUP-d_3, methadone-d_3 and EDDP-d_3 internal standards (IStd) 1 mg/mL or 100 μg/mL ampoules were obtained from Cerilliant (Round Rock, TX, USA). Morphine, codeine, 6AM, 6AC, cocaine, BE, EME, BUP, and methadone quality control (QC) standards, and 6AC-d_3 IStd 1 mg/mL ampoules were obtained from Lipomed (Cambridge, MA, USA). Trans-3′-hydroxycotinine (OH-cotinine) and OH-cotinine-d_3 powder were obtained from Toronto Research Chemicals (North York, ON, Canada). Heroin, nicotine, cotinine, NBUP and EDDP QC samples were prepared from a different lot from Cerilliant, when possible, or from a different vial, with preparation on different days than for calibrators. OH-Cotinine QC samples were prepared on different days than calibrators.

Solid-phase extraction (SPE) was performed with Strata-XC cartridges (60 mg/3 mL; Phenomenex, Torrance, CA, USA). Ultra pure HPLC water, glacial acetic acid, methylene chloride, ammonium hydroxide, urea and sodium hydroxide were obtained from JT Baker (Philipsburg, NJ, USA). 2-isopropanol, ammonium chloride, sodium chloride and lactic acid were purchased from Sigma-Aldrich (St Louis, MO, USA), sodium acetate from EMD Chemicals Inc. (Gibbstown, NJ, USA), and acetonitrile HPLC-grade from Acros (Morris Plains, NJ, USA). All organic solvents were HPLC grade. PharmCheck™ sweat patches were supplied by PharmChem Inc. (Fort Worth, TX, USA).

Artificial sweat solution contained 327 mM ammonium chloride, 166 mM lactic acid, 83 mM urea, 42 mM acetic acid, 34 mM sodium chloride in deionized water; pH was adjusted to 4.7 with 2 mM sodium hydroxide [19].

Instrumentation

LCMSMS analysis was performed with a Shimadzu LC system (Kyoto, Japan) interfaced to a 3200 QTrap (Applied Biosystem/MDS Sciex, Foster City, CA, USA) with an electrospray (ESI) ion source. The Shimadzu system consisted of a binary pump LC-20AD, degasser DGU-20A3, autosampler SIL-20AD and a column oven CTO-20A. Data acquisition, peak integration and calculation were assigned to a computer workstation running Analyst™ Software 1.4.1. Solvent evaporation was carried out on a TurboVap LV evaporator from Zymark (Hopkinton, MA, USA).

Preparation of standard solutions

Working solutions (0.01, 0.05, 0.1, 0.5, 2.5, and 10 μg/mL) of the 14 analytes were prepared by appropriate dilution in methanol. The 1 μg/mL IStd solution was prepared by dilution of 100 μg/mL stock solutions of the 14 deuterated analogs in methanol. Low QC working solution (0.03 μg/mL) for all compounds, except EME (0.15 μg/mL), medium QC working solution (1.5 μg/mL), and high QC working (7.5 μg/mL) were prepared in methanol from different stock solutions than those used for calibrators.

Calibrator and quality control preparation

The pads of new drug-free sweat patches were moistened with 700 μL artificial sweat and dried for 2 h at room temperature (blank sweat patches). Blank sweat patches were spiked with 100 μL working solutions, yielding 1, 5, 10, 50, 250, and 1,000 ng/patch calibrators, 3 ng/patch for low QC for all compounds except EME, 15 ng/patch for EME low QC, 150 ng/patch for medium QC and 750 ng/patch for high QC. After fortification, all patches were allowed to dry at room temperature for 20 min.

Specimen procedure

Sweat patches were folded and placed into 17×60 mm screw-top vials, 6 mL 0.1 M sodium acetate buffer pH 4.5 was added, and vials shaken on a horizontal reciprocating shaker (250 rpm) for 10 min. 3 mL extracting buffer was transferred to a clean tube, spiked with 50 μL of IStd and applied to a Strata-XC cartridge. This cartridge was preconditioned with 2 mL methanol, 2 mL water and 1 mL 0.1 M sodium acetate buffer pH 4.5, and washed with 2 mL water. Vacuum was applied for 10 min to dry the cartridge, and elution achieved with 3 mL dichloromethane:2-isopropanol:ammonium hydroxide (79:20:1, *v/v/v*). The eluate was dried under nitrogen at 45 °C, reconstituted in 100 μL of 0.1% formic acid in water, and 20 μL injected into the LCMSMS.

To document potential hydrolysis of cocaine, 6AM and heroin during sample preparation, blank sweat patches (n= 3 each) were separately fortified with cocaine, 6AM and heroin at the high QC concentration, and the percentage of BE, morphine and 6AM formed were determined.

Liquid chromatography

Chromatographic separation was achieved with a Synergi Polar-RP 80A (75×2 mm, 4 μm) column with a 4×2 mm, identically packed guard column (Phenomenex, Torrance, CA, USA) and gradient elution with mobile phase A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) at a 200 μL/min flow rate. The initial composition (100% A) was maintained for 1 min, B was increased from 0% to 90% over 9.5 min, held at 90% for 1.5 min, and returned to initial conditions over 0.5 min. A 2.5-min equilibration followed, yielding a total run time of 15 min.

Mass spectrometry

Mass spectrometric data were acquired in ESI-positive mode with the following parameters: ionspray voltage, 5,500 V; temperature, 550 °C; curtain gas, 10; ion source gas 1, 50; and ion source gas 2, 55. Curtain and ion source gases were nitrogen. Data were recorded in multiple-reaction monitoring mode. MSMS optimization was established by infusing 0.1 μg/mL of each analyte in methanol directly. The precursor ions, product ions and LCMSMS parameters are displayed in Table 1.

Identification criteria

Identification criteria included retention time (RT) within ±0.2 min of average calibrator RT, presence of 2 transitions, and relative ion intensities (% of base peak) within ±20%, if relative ion intensity was >50%; ±25% if 20–50%; ±30% if 10–20%; and ±50% if ≤10% [20]. These values were compared to mean relative ion intensity of all calibrators.

Validation

Validation parameters included linearity, limits of detection (LOD) and quantification (LOQ), imprecision, analytical recovery, extraction efficiency, process efficiency, matrix effect, selectivity, carryover, dilution integrity, and stability studies. Linearity was

determined by least-squares regression with $1/x^2$ weighting. Acceptable linearity was achieved when the coefficient of determination was at least 0.99 and calibrators quantified within ±20% at the LOQ and ±15% at other concentrations. LOD and LOQ were evaluated with decreasing analyte concentrations in drug-fortified sweat patches. LOD was defined as the lowest concentration with acceptable chromatography, presence of all transitions with signal-to-noise ratios of at least 3, correct relative ion intensities and an RT within ±0.2 min of the average calibrator RT. LOQ was the lowest concentration that met LOD criteria and a signal-to-noise ratio of at least 10, imprecision lower than 20%, and analytical recovery between 80% and 120%.

Imprecision and analytical recovery were determined at three concentrations with five replicates on four different days (n=20). Imprecision, expressed as coefficient of variation (%CV) of the measured values, was expected to be less than 15%. Krouwer and Rabinowitz [21] guidelines were followed for calculation of pooled intra-day, inter-day, and total imprecision. According to the authors [21], within-run imprecision is subtracted from the standard deviation of the mean run averages to obtain a pure between-run component of imprecision. The estimates of imprecision standard deviation within-day (SW), between-day (SB), and total (ST), were:

$$\mathrm{SW}=\sqrt{\text{Mean square within day}}\quad \mathrm{SB}=\sqrt{\text{Means square between day}-\text{Mean square within day}/n}\quad \mathrm{ST}=\sqrt{\mathrm{SW}^2+\mathrm{SB}^2}$$

In the SB equation, n is the number of replicates. It is possible for mean square between-day imprecision to be lower than mean square within day, with the quantity under the square-root sign being negative in this case. If this occurs, SB is set to 0, and inter-day imprecision is then 0. Analytical recovery was evaluated as the percent of target concentration (n=20); analytical recovery was required to be 85–115%, except at the LOQ, when 80–120% was acceptable.

Extraction efficiency for each analyte was measured at each QC concentration. Blank sweat patches were fortified with QC solution before and after patch preparation and SPE. Percent extraction efficiency from sweat patches was expressed as mean analyte area of samples (n=5) fortified with control solution before patch extraction and SPE, divided by mean area of samples (n=5) with control solution added after patch extraction and SPE. Matrix effect was assessed by comparing analyte peak areas in nine different blank extracted sweat patch specimens fortified with QC solutions after patch extraction and SPE, to peak areas of samples at the same nominal concentrations prepared in mobile phase A (neat). These blank sweat patches were worn by healthy non-drug-consuming subjects for 24 h. Matrix suppression or enhancement was calculated as follows: (100×mean peak area of fortified sweat patch after SPE/mean peak area of neat)−100. Process efficiency examined the overall effect of extraction efficiency and matrix effect on quantification of analytes of interest. Mean analyte peak areas of five samples fortified before patch extraction and SPE were compared with mean peak areas of 5 neat samples prepared in mobile phase at the same concentration.

Interferences from endogenous matrix components were evaluated by analyzing sweat patches from nine healthy non-drug-consuming subjects who wore patches for 24 h. IStd was added to patches and they were analyzed as unknowns. Endogenous interferences were considered insignificant if analytes of interest were not detected. Method specificity was demonstrated by adding high concentrations (1,000 ng/patch) of potentially interfering licit and illicit drugs to low QC samples. The following drugs and metabolites were examined: amphetamine, methamphetamine, *p*-hydroxyamphetamine, *p*-hydroxyme-thamphetamine, norephedrine, hydroxynorephedrine, 3,4-methylenedioxymethamphetamine, 3,4-

methylenedioxyam-phetamine, 3,4-methylenedioxyethylamphetamine, 3-hydroxy-4-methoxyamphetamine, 3-hydroxy-4-methoxymethamphet-amine, Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-THC, 11-nor-9-carboxy-THC, diazepam, lorazepam, oxazepam, alprazolam, nitrazepam, flunitrazepam, temazepam, nordiazepam, bromazepam, clonazepam, flurazepam, clonidine, ibuprofen, pentazocine, caffeine, diphenhydramine, chlorpheniramine, brompheniramine, acetylsalicylic acid, acetaminophen and phencyclidine. Sufficient specificity was achieved if analytes of interest quantified within ±15% of low QC concentrations.

Lack of carryover was demonstrated by injecting IStd-fortified blank sweat patches immediately after a sample spiked with all analytes at 2,000 ng/patch, two times the upper LOQ. Carryover was considered negligible if the measured concentration was below the LOD. Dilution integrity was evaluated by diluting the extraction buffer from a sweat patch containing 1,500 ng/patch of each analyte (n=2) with the extraction buffer from a blank sweat patch to achieve 1:10 and 1:50 dilutions. IStd was added to the diluted samples, which were extracted as described. Dilution integrity was maintained if samples quantified within ±15% of 150 (dilution 1:10) or 30 ng/patch (dilution 1:50).

Analyte stability was evaluated for drug-fortified sweat patches stored at room temperature (22 °C) for 24 h, in the refrigerator (4 °C) for 72 h, and after three freeze–thaw cycles (frozen 24 h, thawed kept at room temperature for 3 h). Sweat patches were fortified with analyte mixture 1 (codeine, heroin, cocaine, BUP, methadone) and another set with analyte mixture 2 (morphine, 6AM, 6AC, BE, EME, cotinine, OH-cotinine, NBUP, EDDP) at three concentrations (low, medium, and high QC) in triplicate. Autosampler stability was evaluated injecting reconstituted low, medium and high QC samples stored 48 h at 5 °C on the autosampler. Stability was considered acceptable if QC samples quantified within ±15% of the freshly prepared QC samples.

Method application

The method was applied to the analysis of 16 sweat patch specimens collected once weekly throughout pregnancy from an opioid-dependent pregnant woman receiving controlled BUP treatment. Mean daily dose was 15.6 mg/day. Specimens were collected as part of a protocol providing BUP pharmacotherapy to opioid-dependent pregnant women approved by the Johns Hopkins Bayview and National Institute on Drug Abuse's Institutional Review Boards. The subject provided written informed consent to participate. Specimen collection utilized PharmCheck™ sweat patches (Fort Worth, TX, USA). Sweat patch specimens were collected and stored in the freezer at −20 °C until analysis.

Results

Linearity of analyte-to-IStd peak area ratio versus theoretical concentration was verified in sweat patches with $1/x^2$-weighted linear regression. Curvature tested on a set of four calibration curves yielded determination coefficients (r^2) above 0.99, with residuals within ±20% at LOQs and ±15% at other calibrator concentrations. LODs ranged between 0.5 and 2.5 ng/patch, and LOQs 1 ng/patch, except for EME that was 5 ng/patch. These results are summarized in Table 2.

Imprecision and analytical recovery were satisfactory at all tested concentrations (Table 3). Extraction efficiencies ranged from 35.3% to 160.9%, and process efficiencies from 25.5% to 91.7%. Matrix effect was evaluated for all analytes; ion suppression was noted for EME and EDDP, and ion enhancement for NBUP. Variation for nine different fortified sweat patches was <15%, except for EME and EDDP that showed higher variations (21.2–51.7%). Data are shown in Table 4.

Under the described conditions, no interferences with any extractable endogenous compound in sweat were observed. Method selectivity was demonstrated by adding high concentrations of 35 potentially interfering licit and illicit drugs and metabolites to low QC samples. All analytes quantified within ±15% of target, indicating no interferences with the 14 analytes of interest. No analyte was detected in a blank sample injected immediately following analysis of a 2,000 ng/patch sample, indicating no carryover at this concentration.

The ability of the method to accurately quantify specimens containing high concentrations of analytes was evaluated by diluting buffer from a patch containing 1,500 ng/patch (n=2) 1:10 and 1:50 with buffer used to elute blank sweat patches; 2,700 μL buffer from blank sweat patch was added to 300 μL of buffer from fortified sample to achieve a 1:10 dilution, and 2,940 μL of buffer from blank sweat patch was added to 60 μL buffer from fortified sample to achieve a 1:50 dilution. Samples quantified within 15% of 150 or 30 ng/patch, confirming dilution integrity. Analytes were stable when stored at room temperature (22 °C) for 24 h, refrigerated at 4 °C for 72 h, and after three freeze–thaw cycles, with a % loss<17.4%. Also, analytes were stable in the autosampler at 5 °C for 48 h (% loss<13.7%). Hydrolysis of cocaine, 6AM and heroin during sample preparation was not significant, with BE, morphine, and 6AM concentrations less than the LOQ.

Sixteen weekly sweat patches collected throughout pregnancy from a BUP-treated opioid-dependent pregnant woman were analyzed as a proof of concept of the analytical method. Average sweat patch wear period was 5.9±2.1 days. All positive results obtained are shown in Table 5. No sweat patch was positive for EDDP or codeine. Figure 1 shows the chromatogram of a real specimen positive for BUP, cocaine, opiates and nicotine metabolites.

Discussion

We developed and extensively validated a sensitive (LOD 0.5–2.5 ng/patch) and specific LCMSMS method for the simultaneous quantification of BUP, NBUP, methadone, EDDP, cocaine, BE, EME, morphine, codeine, 6AM, 6AC, heroin, cotinine, and OH-cotinine in a single sweat patch. The assay fulfilled compound identification criteria suggested by the European Union [20] and the Food and Drug Administration [22]. The SAMHSA guidelines for federally mandated sweat testing cutoffs [9] are easily achieved with this method.

Although only one extraction per patch is possible and multiple drug classes could be present in one specimen, most of the published sweat patch methods are focused on a single group of compounds, such as opiates [11, 12], cocaine [13, 14], BUP [15], methadone [16], and nicotine [17]. Kintz et al. developed a GCMS method for the simultaneous determination of opiates, cocaine, cannabis and benzodiazepines [15], and Brunet et al. [7] a GCMS assay for simultaneous determination of cocaine, opiates and methadone. The present method is the first multiple analyte method for drugs in sweat by LCMSMS.

In this method, three additional important opiates were included besides morphine and codeine, i.e. heroin, 6AM and 6AC, specific biomarkers of illicit heroin usage [23– 25]. Kintz [11] included heroin and 6AM, and Brunet [7] heroin, 6AM and 6AC in their methods, but two different extractions [7] or two different injections [11] were necessary to analyze heroin and the other opiates. Also, the method of Brunet et al. [7] had a higher 6AC LOQ (5 ng/patch) than the present method (1 ng/patch).

Kintz et al. [17] developed a method for the quantification of nicotine in sweat patches, but the present method is the first to quantify the main nicotine metabolites, cotinine, and OH-cotinine [26], in sweat. In general, the parent compound is the predominant analyte accumulated in sweat, but detection of nicotine could be due to external contamination [27].

Determination of the metabolites avoids interpretation problems when identifying tobacco exposure. OH-cotinine can only be formed from in vivo nicotine metabolism, however cotinine also may be present in tobacco products, but in low abundance, as a minor alkaloid [26].

All parameters satisfied established criteria, except for EME, EDDP, and NBUP matrix effects. Ion suppression was detected for EME (−63.3%) and EDDP (−60.4%), and enhancement for NBUP (42.6%). Deuterated analogs of each compound were included as IStd, to compensate for these effects. No significant losses were observed for any of the target compounds under the different storage conditions (24 h at 22 °C, 72 h at 4 °C, three freeze/thaw cycles). In accordance with previous publications, Kintz et al. [11] observed no loss of heroin, morphine, and 6AM 1 month at −20 °C, and Follador et al. [13] no cocaine loss over 7 days at room temperature, nor for 14 days at 4 °C.

As proof of concept for the method, 16 sweat patches collected throughout pregnancy from a BUP-maintained opioid-dependent pregnant woman were analyzed. As expected, BUP was detected in a high percentage of specimens (75%), but at low concentrations. Despite receiving a mean daily dose of 15.6 BUP mg/day, maximum BUP concentration was only 2.3 ng/patch, and NBUP was detected in just 6.3% of patches at 1 ng/patch. Kintz et al. [15] reported BUP sweat patch concentrations ranging from 1.3 to 153.2 ng/patch in 16 patients receiving 0.4–6 BUP mg/day, and NBUP in only 1 case with a high BUP concentration (NBUP 3.1 ng/patch, BUP 153.2 ng/ patch). These patches were worn for 5 days. These discrepant findings may be due to differences in sweat excretion rates, since sweat volume production is highly dependent upon an individual's physical activity, emotional state and ambient temperature of the environment [6]. Potential contamination of the patch with BUP cannot be ruled out. Relapse to opiate and cocaine use was detected in 37.5% and 93.8% of specimens, respectively, and methadone diversion was observed in 6.3% of specimens. Cotinine was detected in all specimens with concentrations ranging from 21.4 to 202.0 ng/ patch, indicating tobacco consumption throughout pregnancy. OH-cotinine was detected in 81.3% of specimens, showing less excretion in sweat than cotinine, probably due to its higher polarity.

Conclusion

A highly selective and sensitive LCMSMS method for the simultaneous quantification of BUP, methadone, cocaine, opiates, and nicotine metabolites in sweat was developed and fully validated. This method proved useful for the detection of multiple licit pharmacotherapies for opioid-dependence and for nicotine metabolites, and for identifying relapse to illicit heroin and cocaine.

Acknowledgments

This research was supported by the Intramural Research Program of the National Institute on Drug Abuse (NIDA), National Institutes of Health (NIH), and NIDA grant RO1 DA12220. Sweat patches were generously provided by PharmChem Laboratories, Inc, Fort Worth, Texas, USA.

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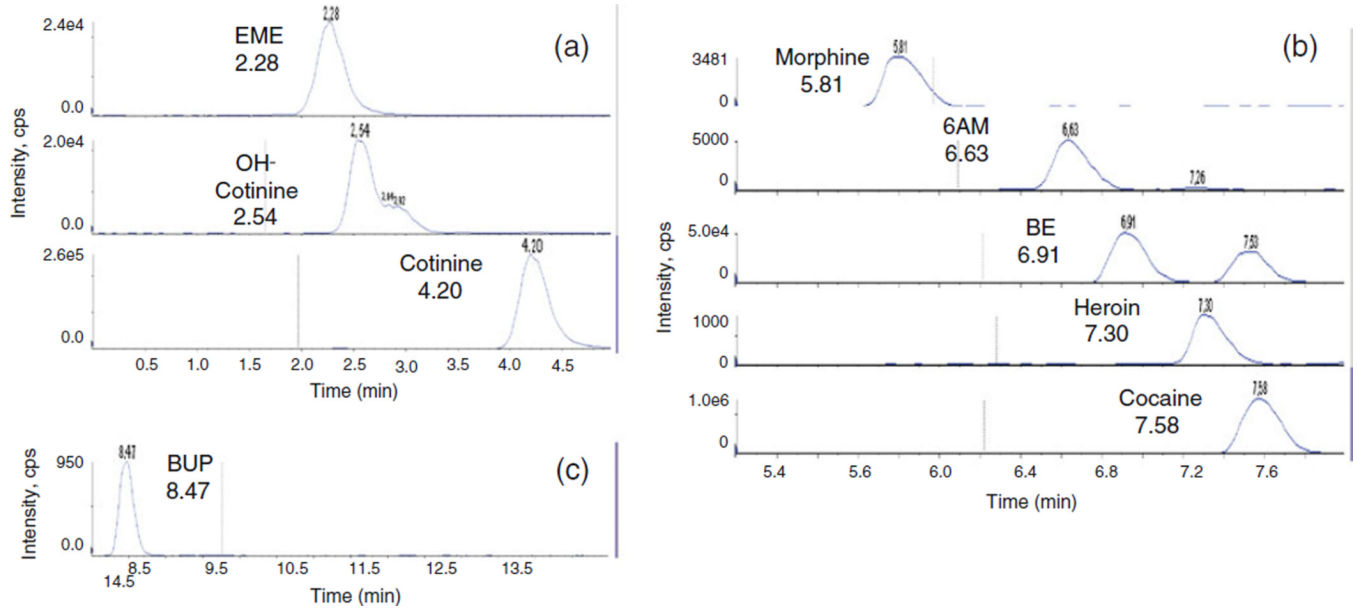

Fig. 1.

LCMSMS chromatogram of a real sweat patch positive to ecgonine methyl ester (EME, 75.5 ng/patch), trans-3′-hydroxycotinine (OH-cotinine, 17.6 ng/patch), cotinine (181.0 ng/patch), morphine (10.1 ng/patch), 6-acetylmorphine (6AM, 4.8 ng/patch), benzoylecgonine (BE, 89.0 ng/patch), heroin (1.4 ng/patch), cocaine (705.0 ng/ patch), and buprenorphine (BUP, 1.6 ng/patch). Quantitation transition of these analytes in the first (a), second (b) and third (c) acquisition windows are included in separate panels

Table 1

LCMSMS parameters, retention times (RT) and internal standards (IStd)

Analyte	MRM transition[a]	DP[b] (V)	EP[c] (V)	CEP[d] (V)	CE[e] (eV)	CXP[f] (V)	RT[g] (min)
EME	*200.2> 182.2*	36	9	12	25	4	2.3
	200.2>82.2	36	9	12	35	4	
EME-d_3	*203.3> 185.3*	41	11	12	25	4	2.3
	203.3>85.2	41	11	12	35	4	
OH-cotinine	*193.2> 80.0*	46	3.5	12	37	4	2.5
	193.2>134.0	46	3.5	12	27	4	
OH-cotinine-d_3	*196.2> 80.0*	46	10.5	14	43	4	2.5
	196.2>134.0	46	10.5	14	27	4	
Cotinine	*177.2> 80.1*	41	10	12	33	4	4.1
	177.2>98.1	41	10	12	29	4	
Cotinine-d_3	*180.2> 80.2*	21	7	12	33	4	4.1
	180.2>101.2	21	7	12	31	4	
Morphine	*286.3> 152.0*	66	3	16	71	4	5.8
	286.3>165.2	66	3	16	51	4	
Morphine-d_6	*292.2> 201.0*	61	3.5	22	25	4	5.8
	292.2>211.1	61	3.5	22	43	4	
Codeine	*300.3> 151.9*	50	6.5	14	91	4	6.4
	300.3>165.1	50	6.5	14	53	8	
Codeine-d_6	*306.3> 152.2*	52	3.5	22	81	4	6.4
	306.3>165.2	52	3.5	22	53	4	
6AM	*328.2> 165.2*	66	7.5	14	53	4	6.6
	328.2>152.2	66	7.5	14	79	4	
6AM-d_6	*334.3 > 165.2*	66	10	16	51	4	6.6
	334.3>152.0	66	10	16	80	4	
BE	*290.2> 168.3*	41	9.5	22	25	4	6.9
	290.2>105.1	41	9.5	22	43	4	
BE-d_8	*298.3> 171.3*	46	3	20	27	4	6.9
	298.3>110.0	46	3	20	41	4	

Analyte	MRM transition[a]	DP[b] (V)	EP[c] (V)	CEP[d] (V)	CE[e] (eV)	CXP[f] (V)	RT[g] (min)
6AC	*342.2> 225.5*	71	4.5	20	41	4	7.3
	342.2>282.2	71	4.5	20	41	4	
6AC-d_3	*345.2> 225.2*	61	6	14	33	6	7.3
	345.2>285.2	61	6	14	27	4	
Heroin	*370.1> 268.2*	76	7	14	39	4	7.3
	370.1 >328.1	76	7	14	35	4	
Heroin-d_9	*379.2> 272.2*	61	3.5	18	39	4	7.3
	379.2>335.3	61	3.5	18	37	4	
NBUP	*414.3> 340.1*	86	12	26	43	4	7.5
	414.3>326.0	86	12	26	40	5	
NBUP-d_3	*417.3 > 343.4*	86	12	16	39	4	7.5
	417.3>326.0	86	12	22	43	4	
Cocaine	*304.3>182.3*	51	3	16	25	4	7.6
	304.3> 82.1	51	3	16	37	4	
Cocaine-d_3	*307.3> 185.3*	41	5	18	27	4	7.6
	307.3>85.2	41	5	18	43	4	
BUP	*468.3> 396.2*	86	12	18	53	6	8.4
	468.3>414.3	86	12	18	45	12	
BUP-d_4	*472.5> 400.1*	86	11	24	61	6	8.4
	472.5>415.3	86	11	24	45	8	
EDDP	*278.2>234.1*	61	4	16	43	4	8.8
	278.2> 249.2	61	4	16	33	4	
EDDP-d_3	*281.3> 234.1*	61	9.5	24	31	4	8.8
	281.3>249.2	61	9.5	24	31	4	
Methadone	*310.3>265.2*	31	4	14	19	4	8.9
	310.3> 223.3	31	4	14	27	4	
Methadone-d_3	*313.3> 268.1*	31	4.5	16	19	4	8.9
	313.3>226.2	31	4.5	16	27	4	

MRM Multiple-reaction monitoring

[a] The quantification transition is rendered in italics

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[b] Declustering potential

[c] Entrance potential

[d] Collision cell entrance potential

[e] Collision energy

[f] Collision cell exit potential

[g] Retention time

Table 2

Limits of detection (LOD), limits of quantification (LOQ) and linearity results

Analyte	LOD (ng/patch)	LOQ (ng/patch)	Linearity (ng/patch)	Intercept±SD (*n*=4)	Slope±SD (*n*=4)	r^2±SD (*n*=4)
EME	2.5	5	5–1000	−0.0013±0.0025	0.0112±0.0010	0.9972±0.0023
BE	0.5	1	1–1000	0.0016±0.0008	0.0071±0.0004	0.9926±0.0013
Cocaine	0.5	1	1–1000	0.0003±0.0001	0.0019±0.0001	0.9964±0.0013
Morphine	0.75	1	1–1000	0.0058±0.0025	0.0381±0.0044	0.9936±0.0030
Codeine	0.75	1	1–1000	0.0004±0.0013	0.0101±0.0003	0.9963±0.0032
6AM	0.75	1	1–1000	0.0004±0.0018	0.0114±0.0006	0.9991±0.0004
6AC	0.75	1	1–1000	0.0009±0.0007	0.0077±0.0004	0.9967±0.0002
Heroin	0.5	1	1–1000	0.0010±0.0005	0.0104±0.0002	0.9978±0.0002
Cotinine	0.75	1	1–1000	0.0067±0.0019	0.0115±0.0006	0.9962±0.0017
OH-Cotinine	0.75	1	1–1000	0.0008±0.0007	0.0073±0.0002	0.9977±0.0012
Methadone	0.5	1	1–1000	0.0001±0	0.0014±0.0001	0.9929±0.0008
EDDP	0.5	1	1–1000	0.0015±0.0003	0.0088±0.0006	0.9928±0.0028
BUP	0.5	1	1–1000	0.0001±0.0013	0.0182±0.0007	0.9980±0.0005
NBUP	0.75	1	1–1000	0.0047±0.0024	0.0074±0.0003	0.9967±0.0019

Table 3

Pooled intra-, inter-, and total imprecision and analytical recovery

Analyte	Pooled intra-day imprecision (*n*=20, CV)			Inter-day imprecision (*n*=20, CV)			Total imprecision (*n*=20, CV)			Analytical recovery (*n*=20,% target)		
	Low	Med	High	Low	Med	High	Low	Med	High	Low	Med	High
EME	6.9	1.7	3.3	7.4	6.4	2.4	10.1	6.6	4.1	96.2	90.4	90.5
BE	5.8	3.7	5.0	0.6	6.7	1.7	5.8	7.6	5.3	107.7	100.3	99.6
Cocaine	7.0	2.6	2.3	7.5	8.7	2.7	10.3	9.0	3.5	98.5	91.7	93.8
Morphine	6.2	6.7	5.2	3.4	3.4	4.6	7.1	7.6	7.0	92.4	93.7	94.3
Codeine	5.2	3.2	3.7	8.3	4.1	0.7	9.8	5.2	3.6	94.3	91.2	90.1
6AM	2.8	2.9	3.8	1.5	6.6	2.8	3.2	7.2	4.7	91.6	90.9	94.2
6AC	3.6	1.7	1.6	8.7	8.8	2.7	9.4	8.9	3.1	96.2	93.9	97.3
Heroin	3.9	2.4	1.9	2.1	5.3	2.0	4.4	5.8	2.3	92.9	91.3	91.1
Cotinine	7.2	3.7	3.8	3.0	3.7	3.9	7.8	5.2	5.5	96.8	92.6	92.1
OH-Cotinine	5	3.2	4.3	1.1	7.2	1.2	5.1	7.9	4.4	96.1	92.2	95.2
Methadone	3.9	1.7	2.0	9.1	3.0	0	9.9	3.5	0	95.6	90.1	87.9
EDDP	3.2	2.1	2.4	1.3	2.7	3	3.5	3.4	3.8	92.4	90.9	87.2
BUP	2.7	2.5	2.1	9.3	1.5	1.9	9.7	3.0	2.8	97.2	92.5	90.6
NBUP	6.2	2.3	2.5	4.0	7.1	3.0	7.4	7.4	3.9	98.1	92.2	93.9

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Table 4

Extraction efficiency, process efficiency and matrix effect

Analyte	Extraction efficiency (*n*=5)			Process efficiency (*n*=5)			Matrix effect (CV%) (*n*=10)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
EME	56.9	70.8	160.9	28.3	37.2	52.6	−53.3 (13.9)	−63.3 (51.7)	−63.3 (29.3)
BE	35.3	36.9	46.7	41.3	38.6	43.8	14.8 (2.7)	2.8 (3.0)	−7.7 (2.3)
Cocaine	38.8	45.8	47.7	32.5	42.3	46.7	−14.1 (4.5)	−9.3 (3.4)	−3.7 (2.9)
Morphine	40.6	45.0	42.2	32.4	40.8	40.6	−19.2 (6.9)	−11.9 (5.8)	−6.0 (3.1)
Codeine	42.8	46.4	48.7	42.9	43.2	46.6	1.8 (3.3)	−8.4 (3.7)	−6.4 (3.4)
6AM	37.3	41.8	44.7	38.0	40.3	46.4	4.7 (4.7)	−5.6 (7.4)	1.8 (3.5)
6AC	38.5	46.1	51.2	35.5	42.2	49.4	−8.2 (3.4)	−9.7 (3.4)	−5.5 (3.0)
Heroin	46.2	53.6	54.7	54.4	51.7	48.9	16.1 (5.6)	−6.3 (9.3)	−12.4 (3.7)
Cotinine	49.1	50.9	50.6	49.3	51.9	53.3	5.6 (8.1)	−3.8 (7.6)	4.4 (3.2)
OH-Cotinine	39.2	45.6	45.7	37.4	45.4	44.7	−4.3 (3.0)	0.02 (2.6)	−3.6 (2.7)
Methadone	54.5	54.1	58.4	46.2	56.3	62.3	−10.5 (9.8)	−0.6 (7.6)	4.1 (6.6)
EDDP	63.5	51.3	52.2	25.5	25.7	49.5	−56.5 (21.2)	−60.4 (36.0)	−9.5 (7.6)
BUP	40.4	46.0	47.9	45.2	47.5	49.6	12.7 (2.4)	1.5 (2.9)	0.3 (4.5)
NBUP	65.4	54.4	52.8	91.7	55.7	50.5	42.6 (4.2)	−2.1 (7.5)	−7.0 (4.3)

Table 5

Drug concentrations in 16 sweat patches worn an average 5.9±2.1 days throughout pregnancy from one opioid-dependent pregnant woman receiving buprenorphine-assisted pharmacotherapy (mean daily dose 15.6 mg/day)

Analyte	Number of positive sweat patches	Median (ng/patch)	Range (ng/patch)
EME	9	11.6	10.9–75.5
BE	9	14.5	12.7–230
Cocaine	15	90.6	1.0–1420
Morphine	6	0	2.3–10.1
6AM	4	0	2.4–44.9
6AC	1	0	17.8
Heroin	3	0	1.4–526
Cotinine	16	78.5	21.4–202
OH-Cotinine	13	6.6	3.1–17.6
Methadone	1	0	1.0
BUP	12	1	1–2.3
NBUP	1	0	1.0