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The Current Status of Sweat Testing For Drugs of Abuse: A Review

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Abstract: Sweat is an alternative biological matrix useful to detect drugs of abuse intake. It is produced by eccrine and apocrine glands originating in the skin dermis and terminating in secretory canals that flow into the skin surface and hair follicles. Since many years it has been demonstrated that endogenous and exogenous chemicals are secreted in this biological sample hence its collection and analysis could show the past intake of xenobiotics. From the seventies the excretion of drugs of abuse has been investigated in human skin excretion; later in nineties forensic scientists began to experiment some techniques to trap sweat for analyses. Even if the use of skin excretions for drug testing has been restricted mainly by difficulties in sample recovery, the marketing of systems for the sample collection has allowed successful sweat testing for several drugs of abuse. In the recent years sweat testing developed a noninvasive monitoring of drug exposure in various contexts as criminal justice, employment and outpatient clinical settings. This paper provides an overview of literature data about sweat drug testing procedures for various xenobiotics especially cocaine metabolites, opiates, cannabis and amphetamines. Issues related to collection, analysis and interpretation of skin excretions as well as its advantages and disadvantages are discussed. Moreover the chance to apply the technique to some particular situation such as workplace drug testing, drivers, doping or prenatal diagnosis, the comparison between sweat and other non conventional matrices are also reviewed. According to literature data the analysis of sweat may be usefully alternative for verifying drug history and for monitoring compliance.

Keywords: Sweat testing, drugs of abuse, unconventional matrix.

1. INTRODUCTION

The diagnosis of acute intoxication by xenobiotics, together with the determination of drug use/abuse is the target of forensic toxicology. Analysis of biological fluids and tissues provides the most objective method for documenting human drug exposure. The choice of biological matrices is the crucial step for a correct investigation, because each biological specimen is unique and offers a somewhat different pattern of information regarding drug use over time. The toxicologist needs a deep knowing of various parameters such as the purpose of the investigation, the kind of substances to be identified, the time, way and modality of intake, the knowledge of pharmacokinetic and pharmacodynamic of drugs [1].

Blood and urine are historically the biological matrices more employed for testing of drugs of abuse both of cadavers and living people. However, currently there is growing interest in the use of alternative body fluids and tissues such as saliva, skin excretions and hair for the diagnosis of drug use [2-7]. The purpose of these studies was the exploration of less invasive collectors in order to obtain more information regarding the use/abuse of psychotropic drugs. Research may involve the detection of the parent compound or metabolites and sensitivity, specificity, and reliability of drug testing may vary depending on the drug [7]. Scientific international literature developed analytical methodologies to detect xenobiotics on sweat, matrix in which illicit compounds can be found with a time window that allows peculiar information different from other biological samples [8].

As referred by Kintz P. [9] since 1911 researchers established that drugs are excreted by the body in sweat, but many analytical and practical problems mainly due to the difficulty in collecting skin excretions, did not allow its application in forensic toxicology until 1990s. In 1980 Phillips M. [10] devised an occlusive adhesive patch that trapped solute and water components in sweat providing a possible means to monitor patient compliance with therapeutic regimens. The patch consisted of an absorbent pad impregnated with sodium chloride crystals under a water-proof dressing. Later, occlusive bandages, consisting of one to three layers of filter papers or pieces of cotton, gauze, or towel were proposed to collect sweat [9, 11]. Significant advances have been made in the past years to develop a sweat-patch technology. In fact a non occlusive sweat collection device (patch) was developed by a commercial firm (Sudormed, Santa Ana, CA, USA) in 1990.

The variation between individuals in the amount of sweat they excreted has caused difficulty for those attempting to construct a universal sweat collection device. Earlier experiments to test for the presence of specific substances in sweat have used patches that occlude the skin causing numerous problems such as skin irritation, alteration of both the steady-state, pH of the skin and the skin's colonizing bacteria [12]. Newer non occlusive patches use a transparent film that allows oxygen, carbon dioxide and water vapor to escape, while trapping the necessary traces of drug use excreted in sweat [13]. Cone E.J. [8] found many benefits in using this type of patch, including high subject acceptability, low incidence of allergic reactions to the patch adhesive and ability to monitor drug intake for a period of several weeks with a single patch. Several studies have also found that the patch is resistant to inconspicuous tampering [8, 12, 14]. Kintz P. *et al.* [12] also reported that no special precautions were needed to wear the patches for several days except to avoid excessive towel rubbing after bathing. Hence, success in sweat testing for several drugs of abuse has been accomplished because of substantial advances in sample collection and improved accuracy of measurement methods. Consequently remarkable advances in sensitive analytical techniques have enabled the analysis of drugs in unconventional samples such as skin excretions.

Some reviews regarding the employ of alternative biological matrices were published in different periods. The first paper found in literature [15] referred about the detection of drugs of abuse in hair, nail, saliva and sweat. Preparation or pretreatment of samples, analytical procedures, and the interpretation of analytical results are discussed concomitantly.

A monographic report of NIDA was published in the year 1997 by Cone E.J. [8]. The usefulness of various biological fluids, including sweat, together with the chemical and physical properties was discussed. Research of sweat testing for drugs had been limited because of the difficulty in collecting sweat samples and the author suggests the employ of a sweat collection device that appeared to offer promise for the collection of this sample. He refers about the advantages of the sweat patch for drug monitoring that include the high subject acceptability of wearing the patch, low incidence of allergic reactions to the patch adhesive and ability to monitor drug intake for a period of several weeks for the single patch.

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A review referring on the detection of marijuana, cocaine**,** opiates, amphetamines, benzodiazepines, barbiturates, phencyclidine and nicotine in sweat is reported with emphasis on forensic applications [16]. Sweat maybe applicable for use in driving while intoxicated and surveying populations for illicit drug use. The review refers about advantages and disadvantages of sweat testing compared to saliva and urine.

Huestis MA *et al.* [11] in 1999 reviewed the detection of cocaine, codeine and metabolites in sweat by GC/MS detailing results from a new type of sweat collection device that allows rapid collection of sweat samples.

Various aspects concerning the practical application and forensic interpretation of data obtained by drug monitoring from the skin surface are discussed by Skopp G. *et al.* [17]. Basic information on the composition of skin secretions and their particular transport mechanisms, as far as known, are given. Drug molecules from blood are considered to reach the skin surface by various routes such as by sweat and sebum as well as by inter- and/or transcellular diffusion. The role of the stratum corneum as a temporary drug reservoir exceeding positive drug findings in urine is outlined.

Cone E.J. in 2001 [18] provided an overview of global drug trafficking patterns and drug use, and results from a survey of legal statutes in twenty countries covering use of alternate matrices for drug testing. He stated that advances have also been made in the use of alternate biological matrices such as sweat for drug testing. Dolan K. *et al.* [19] published a brief overview providing qualitative drug testing procedures using sweat; authors stated that sweat may be useful in the detection of illicit drug use for developments in patch technology which allows for a cumulative estimate of drug exposure over several days. In the year 2010 Maurer H.H. [20] published an important review on analytical toxicology describing the procedures for screening, identification and quantification of drugs, poisons and their metabolites in various matrices including sweat. The paper focused on the selection of the most appropriate bio-sample to be analyzed depending of the task to be fulfilled.

The authors of the present review believe useful to briefly refer about the employ of sweat in clinical settings. Shearer D.S. *et al.* in 1998 [21] performed a drug testing of patients in a psychiatric outpatient service allowing to identify patients who relapse into renewed use of drugs of abuse and in monitoring the effectiveness of ongoing medical and psychological therapy. Moreover DuPont RL. [22] proposed sweat for clinical settings and affirmed it could be useful in schools and in-family based efforts to prevent drug use.

This review focuses the attention on skin excretions that may provide an additional tool for monitoring drug use. Although the use of sweat for drug testing has been hampered by difficulties in sample collection and sensitivity of analytical methods, successful sweat testing for several drugs of abuse has been accomplished because of substantial advances facilitating sample collection and improving the accuracy of diagnostic techniques [8].

For this purpose a computerized search of articles inserted in "PUBMED" and "SCOPUS" from 1992 to 2011 was performed. 100 papers were chosen including some reviews for their relevance on sweat technology (Table **1**). The articles reviewed were scheduled on the basis of the population studied and/or the referred application (clinical setting, forensic application, workplace drug testing, pregnancy, controlled administration, roadside testing, etc).

2. HUMAN SWEAT

Human sweat is a biological fluid and its secretion is an important homeostatic mechanism for maintaining a constant core body temperature to a narrow physiological range [11]. Randall W.C. in 1953 [23] stated that at temperatures above 31°C body heat is dissipated by the release of sweat on the skin surface resulting in evaporative heat loss however a loss by sweating can also occur at other temperatures. Eichna L.W. [24] asserted that the amount of sweat secreted is highly variable and dependent upon daily activity, emotional state and environmental temperature. Sweat is eliminated from human body through the skin that consists of two layers; the outer epidermis and the inner dermis. The epidermis in turn is composed of two main cell types, the pigmented melanocytes which protect against damaging effects of sunlight, and keratocytes which contain the filaments that provide the structural integrity of the skin. As keratocytes mature they lose their cell nucleus and move to the outer portion of the skin; hence this layer (called stratum corneum) consists entirely of keratocytes that have lost their nuclei. The underlying dermis, which makes up the bulk of the skin is composed of fibrous well vascularized connective tissue and it contains the hair follicles, sweat and sebaceous glands [25]. Under the dermis there is the adipose layer that consists of lobules of fat separated by fibrous connective tissue. Blood vessels pass into and through this layer. Sweat is approximately 99% water with the most concentrated solute being sodium chloride. The rate of sweating is highly dependent upon environmental temperatures and rates as high as 3 l/min have been recorded for short periods [2]. The majority of sweat is produced by eccrine glands located in the transdermal layer of most skin surfaces. Apocrine glands are an other type of sweat gland located in specific regional areas like the skin of the axilla, pubic region and around the nipples. Sweat glands often develop in close association with hair follicles and sometimes empty directly into hair follicles. Approximately 50% of the total volume of sweat is produced by the trunk, 25% by the legs and 25% by the head and upper extremities [2]. Besides aqueous secretion, the skin is also bathed with sebaceous secretions especially on the face and scalp. The sebaceous secretions are primarily lipids that may transport and adsorb many drugs [16].

Sweat and sebaceous glands are housed in the dermis and are distributed through the body disproportionately. The highest concentration of sweat glands resides in the hands, while the forehead contains the densest population of sebaceous glands [26]. Moisture maybe lost from the skin by either insensible sweat likely caused by diffusion through the skin and sensible sweat which is actively excreted during stress and exercise [16]. Several reports have demonstrated the sweat is suitable alternative biological matrix for monitoring recent drug use because a small but sufficient fraction of the drug is excreted by the skin [8, 20, 25].

3. EXCRETION OF XENOBIOTICS INTO SWEAT

The excretion of drugs in sweat has important implications in clinical and forensic toxicology as well as in preventative medicine. Specific and sensitive detection or precise quantification of xenobiotics in bio-samples are great challenges in analytical toxicology. Investigators have been studying the secretion of endogenous and exogenous chemicals in sweat for many years. The sebaceous secretion is primarily constituted by lipids that may transport and absorb many drugs. Different concentrations of drugs may be expected, depending upon the area of the body in which the sample is taken, because fat-soluble drugs may be sequestered or secreted by the skin. The mechanism by which drugs are incorporated into sweat is not fully understood [27] and there are several potential mechanisms by which drugs may be secreted in sweat including passive diffusion from blood into sweat glands and transdermal migration of drugs across the skin [2]. Non-ionized basic drugs diffuse into sweat and become ionized as a result of the lower pH of sweat as compared to blood [28]. The pH of sweat is generally in the range of 4 to 6.8, with the average sweat pH from resting individuals considered to be 5.8. With the increased flow rate (following exercise or above 31°C), sweat pH has been found to increase to 6.8 [8, 28]. A low basal pH should favor concentration of basic drugs in sweat thus producing a free-drug sweat/plasma (S/P) ratio greater than 1. This assumption is supported by studies of the excretion of ammonia in sweat. The observed S/P ratios for total ammo-

Table 1. Drugs of Abuse in Sweat: Crucial Aspects of Methodology

Reference	Author	Drugs and Metabolites	Collection Device	Modality and Time of Wear	Analytical Method	Other Matrices	Application
[2]	Cone EJ. *et al.* 1994	heroin and cocaine	Sudormed/Band-aid	Back, Abdomen / 0-3 Days	GC/MS	urine, hair	Clinical setting
[3]	Smith FP. *et al.* 1996	cocaine	Sweat wipes	forehead skin swabs	RIA-GC/MS	hair, saliva, urine	Drug users and children
[4]	Kidwell DA. *et al.* 1997	cocaine	Sweat wipes	wiping forehead	GC/MS/MS	hair	University population
[5]	De Oliveira CDR. *et al.* 2007				GC/MS LC/MS	saliva, hair, nails,	Review of chromatographic procedures
[7]	Vearrier D. *et al.* 2010					various	Review of biological matrices
[8]	Cone EJ. 1997						Monography
[9]	Kintz P. 1996	opiates, cocaine, cannabinoids, buprenorphine, metadone, nordiazepam	PharmChek	back / 1 week	GC/MS	urine, hair	Clinical setting
[11]	Huestis M. *et al.* 1999	cocaine , codeine	Sudormed/Fast Patch	palm, torso / 30 minutes	GC/MS		Review of sweat testing
[12]	Kintz P. el al 1997	opiates	PharmChek	back /24 hours	GC/MS	no	Clinical setting
[13]	Kidwell DA. *et al.* 2001	cocaine, metamphetamine, heroin	PharmCheck	arms	GC/MS	no	Environmental contamination
[14]	Caplan YH. *et al.* 2001					hair, nail, blood , urine	Workplace drug testing
[15]	Inoue T. el al 1992						Review of biological matrices
[16]	Kidwell DA. *et al.* 1998					saliva	Review - Forensic
[17]	Skopp G. *et al.* 1999					saliva	Review- Roadside testing
[18]	Cone EJ. 2001						Review- Workplace drug testing
[19]	Dolan K. *et al.* 2004					urine, hair, saliva	Review- Forensic
[20]	Maurer HH. 2010					urine, blood, tissues, hair, oral fluid, nails, meconium	Review of analytical toxicology
[21]	Shearer DS. *et al.* 1998					urine,saliva, hair, urine	Review of biological matrices, Psychiatric
[22]	DuPont RL. 2010					hair, saliva, urine	General aspects, Clinical setting
[25]	Levisky JA *et al.* 2000	cocaine, opiates	Adipose tissue. skin	skin collected during autopsy	GC/MS	blood	Autoptical data
[26]	Chawarski MC *et al.* 2007	opiates	PharmCheck	not specify	GC/MS	urine	Clinical setting
[27]	Brunet BR *et al.* 2010	cocaine, opiates	PharmCheck	back. arm /1 week	GC/MS	no	Pregnancy
[28]	Huestis MA etal 1998					alternative matrices	Monography
[30]	Kacinko S.L. *et al.* 2005	cocaine	PharmCheck	back, abdomen / 4-15 hours	GC/MS	no	Controlled administration
[31]	Uemura N. *et al.* 2004	cocaine	PharmCheck	back, shoulder / 1-72 hours	GC/MS	no	Controlled administration
[32]	Schwilke EW.*et al.* 2006	opiates	PharmCheck	abdomen, back / 1 week, 1-15 hours	GC/MS	no	Controlled administration
[34]	Faergermann J. *et al.* 1993	terbinafine	stratum corneum, dermis-epidermis, sebum			hair, nails, plasma	Controlled administration
[35]	Burns M. *et al.* 1995	cocaine	Band-aid	torso, biceps, back / 1 week		urine	Clinical setting

(Table 1) contd…

Reference	Author	Drugs and Metabolites	Collection Device	Modality and Time of Wear	Analytical Method	Other Matrices	Application
[36]	Joseph RE. *et al.* 1998	cocaine, opiates	Sebutape	back, forehead /1-2 hours	GC/MS	plasma, sebum, stratum corneum	Controlled administration
[37]	Liberty HJ. *et al.* 2004	cocaine	PharmCheck	biceps/ various time	GC/MS	urine	Controlled administration
[38]	Kidwell DA. *et al.* 2003	cocaine	PharmChek-Skin swabs	arms /1-4 weeks	immunoassay (CEDIA, Cozart,OraSure ELISA) - GC/MS	urine	Environmental contamination
[39]	Pichini S. *et al.* 2003	MDMA	PharmCheck-Drugwipe	24 hours (back)	immunoassay - GC/MS	urine	Controlled administration
[40]	Balabanova S. *et al.* 1992	cocaine, morphine, methadone	Pilocarpine stimulation		RIA		Drugs users
[42]	Spiehler V. *et al.* 1996	cocaine	PharmChek	skin / 7 days	Immunoassay-GC/MS	no	Drugs users, controlled administration
[43]	Burns M. *et al.* 1995	cocaine	PharmChek/Band-aid	up to 7 days	RIA-GC/MS	no	Controlled administration
[44]	Skopp G. *et al.* 1996	theophylline, methadone, heroin , cocaine	Sudormed				Controlled administration
[45]	Kintz P. *et al.* 1996	diazepam	Sudormed	0-72 hours	GC/MS	no	Controlled administration
[46]	Kintz P. *et al.* 1996	codeine, phenobarbital	Sudormed	different sites / up to 7 days	GC/MS		Controlled administration
[47]	Liberty HJ. Et al 2003	crack	PharmChek, Fastpatch	one per hand /15-30 minutes	GC/MS	urine	Controlled administration
[48]	Fogerson R. *et al.* 1997	opiates	PharmChek	skin /1-10 day	EIA-GC/MS	urine	Controlled administration, adulteration study
[50]	Moody DE. *et al.* 2001	cocaine, heroin	PharmChek		RIA /EIA	no	*In vitro* study
[51]	Moody DE. *et al.* 2004	cocaine	PharmChek		RIA/GC/MS		Controlled administration
[52]	Mura P. *et al.* 1999	cannabinoids	Drugwipe		GC/MS	urine,saliva,tears	Drug users and drug free
[53]	Samyn N. *et al.* 2000	amphetamines, cocaine, opiates, cannabis	Drugwipe		GC/MS	saliva, plasma, urine	Drug users
[54]	Pacifici R. *et al.* 2001	MDMA	Drugwipe		GC/MS	plasma, urine	Controlled administration
[55]	Hazarika P. *et al.* 2010	cocaine, opiates	Fingermark		immunoassay-Fluorescence microscopy		Drug users
[57]	Fay J. *et al.* 1996	metamphetamine	PharmChek		EIA-GC/MS		Controlled administartion
[58]	Maurer HH. *et al.* 1997	xenobiotics			GC/MS-LC/MS	blood, urine, saliva, hair	General aspects on analytical toxicology
[59]	Segura J. *et al.* 1998						General aspects on analytical toxicology
[60]	Kintz P. *et al.* 1998	nicotine	PharmChek	72 hours	GC/MS		Cigarettes smokers and nonsmokers
[61]	Preston KL. *et al.* 1999	cocaine	sweat patches		ELISA- GC/MS	urine	Clinical setting
[62]	Huestis M. *et al.* 2000	opiates	PharmChek	abdomen, back/ 1 week	ELISA- GC/MS	urine	Clinical setting
[63]	Kintz P. *et al.* 2002	flunitrazepam, GHB, cannabinoids, LSD, ecstasy, ethanol			GC/MS/MS/NICI		Crime under the influence
[64]	Samyn N. *et al.* 2002	cocaine, amphetamines, cannabis	Drugwipe		GC/MS	blood, urine, oral fluid	Roadside testing

(Table 1) contd…

Reference	Author	Drugs and Metabolites	Collection Device	Modality and Time of Wear	Analytical Method	Other Matrices	Application
[65]	Saito T. *et al.* 2004	cannabinoids	PharmChek	skin /12 hours	GC/MS-NICI	no	Method validation, clinical setting
[66]	Follador MJ. *et al.* 2004	cocaine, cocaetilene	PharmChek	sweating part of the body / 3-7 days	GC/MS	no	Method validation
[67]	Pichini S.*et al.* 2005	salvia divinorum		back / 2 hours	GC/MS	blood, urine, saliva	Controlled study
[68]	Yang W. *et al.* 2006	metamphetamine, cocaine, codeine	skin biopsy (gluteus maximus)		GC/MS	no	Clinical setting - Method validation
[69]	Abanades S. *et al.* 2007	GHB	PharmChek	back/ 6 hours	GC/MS	plasma, oral fluid, urine	Controlled administration- Pharmacokinetic
[70]	De Martinis BS. *et al.*2007	amphetamines analogs	PharmChek		GC/MS	no	Method validation , *in vitro* study, controlled administration
[71]	Brunet BR. *et al.* 2008	methadone, heroin, cocaine	PharmChek	not specified / 7 days	GC/MS		Method validation, pregnancy
[72]	Fucci N.*et al.* 2008	methadone	PharmChek	upper arms / 7 days	GC/MS	hair, urine	Clinical setting
[73]	Barnes Aj. *et al.* 2009	MDMA	PharmChek	back, abdomen / 2 hours - 7 days	GC/MS	no	Controlled administration
[74]	Barnes AJ. *et al.* 2010	methadone	PharmChek	back, arm / 2-24 days	GC/MS	no	Controlled administration, pregnancy
[75]	Concheiro M. *et al.* 2011	buprenorphine, methadone, cocaine, opiates, nicotine	Band-aid	back, upper arm, lower chest / 7 days	LC/MS/MS	no	Method validation
[76]	Marchei E. *et al.* 2010	methylphenidate	PharmChek	back/ 24 hours	LC/MS	oral fluid, plasma	Pilot study
[77]	Cirimele V. *et al.* 2000	clozapine			LC/MS	plasma, hair	Schizophrenic patients
[78]	Al-dirbashi OY. *et al.*2001	metamphetamine, amphetamine	Abusers' clothes		HPLC/ UV - fluorescence detection		Method validation
[79]	Crouch DJ. *et al.* 2001	amphetamine, cannabinoids, cocaine, opiates, PCP	Macroduct (pilocarpine stimulation)		screening LC/MS/MS	urine	Pilot study
[80]	Kintz P. *et al.* 1998	methadone	PharmCheck	upper back (72 hours)	LC/MS	urine	Clinical setting
[81]	Samyn N. *et al.* 2002	ecstasy	SweatWipe	wiping with cotton over forehead	GC/MS	plasma, oral fluid, urine	Controlled administration
[82]	Gallardo 2009						Workplace drug testing
[83]	Marchei E. *et al.* 2012	atomoxetine		back / 6 hours	LC/MS/MS	blood, urine, oral fluid	Controlled study
[84]	Kintz P. 1996	opiates, cocaine, cannabinoids, benzodiazepine, amphetamine, buprenorphine	PharmCheck	5 days	GC/MS-LC/MS	urine	Drug users
[85]	Taylor JR. *et al.* 1998	methadone, cocaine, opiates	PharmCheck	5-10 days	Immunoassay	urine	Clinical setting
[86]	Levisky JA. *et al.* 2001	cocaine, metamphetamine	sweat patches	10 -14 days	GC/MS	urine	Drug users
[87]	De la torre R. *et al.* 2004	amphetamines					Review- Pharmacokinetic study
[89]	Barnes AJ. *et al.* 2008	metamphetamine, amphetamine	PharmCheck	back, abdomen / 1 week	GC/MS	no	Controlled administration
[90]	Kintz P. 1997	MBDB, BDB	PharmCheck	upper arm / up to 72 hours	GC/MS	urine	Controlled administration
[91]	Kintz P. *et al.* 1999	ecstasy					Review biological matrices
[92]	De Martinis BS. 2008	amphetamines					Review of the scientific literature

(Table 1) contd…

Reference	Author	Drugs and Metabolites	Collection Device	Modality and Time of Wear	Analytical Method	Other Matrices	Application
[93]	De la torre R. *et al.* 2004	cannabinoids					Review
[95]	Staub C. 1999	cannabinoids				blood, saliva, hair, meconium	Review of chromatographic procedures
[96]	Kintz P. *et al.* 2000	cannabinoids	forehead wipes (cosmetic pad)		GC/MS	blood, urine, oral fluid	Roadside testing
[97]	Huestis M. *et al.* 2008	cannabinoids	PharmChek	Chest, abdomen /1-7 days	GC/MS		Pharmacokinetic study
[98]	Winhusen TM. *et al.* 2003	cocaine	PharmCheck	right arm, left arm / 7 days	GC/MS	urine	Clinical setting
[99]	Kintz P. 1998	codeine	Drugwipe-PharmCheck	wiping forehead- upper arm	GC/MS	saliva	Controlled administration
[101]	Concheiro M. *et al.* 2011	buprenorphine	PharmCheck	12-24 hours	GC/MS	plasma, oral fluid	Controlled administration in pregnancy
[102]	Balabanova S. *et al.* 1995	nicotine	pilocarpine stimulation	sweat taken every hour for 6 hours	RIA/GC/MS		Smokers and no smokers subjects
[104]	Marchei E. *et al.* 2010	methylphenidate	PharmChek	up to 24 hours		oral fluid	Pediatric subjects, pharmacokinetic study
[105]	Lankheet N.A. *et al.* 2011	sunitinib and metabolites	PharmChek	upper arm /24 hours	LC/MS/MS	no	Method validation - Clinical setting
[107]	Samyn N. *et al.* 2000	amphetamines			on-site testing	urine, saliva	Roadside testing
[108]	Kintz P. *et al.* 2000						Roadside testing- General aspects
[109]	Walsh JM. *et al.* 2004						Roadside testing - General aspects
[110]	Rivier L. 2000						Review- Alternative biological samples
[111]	Rivier L. 2000						Doping- Alternative biological samples
[112]	Huestis M.*et al.* 2002						Review- Pregnancy , alternative biological samples
[113]	Lozano J. *et al.* 2007						Review- Pregnancy , alternative biological samples
[114]	Gray T. *et al.* 2007						Review- Pregnancy , alternative biological samples
[115]	Daughton CG. 2011						Review- Forensic

nia have been reported to be 20-50 [2]. The stratum corneum contains structures that may function as diffusion shunts, thus rendering three potentially distinct routes of penetration through the stratum corneum: hair follicles, sweat ducts and the unbroken stratum corneum. The study on steady state drug transport through the skin support the contention that bulk diffusion pathway through the intact stratum corneum predominates over diffusion shunts. Delivery of high concentrations of the drug to the skin surface by sebum and sweat could produce a deposition on the stratum corneum and allow the skin to serve as a shallow drug depot [11, 29]. Many illicit drugs may diffuse through the dermal and epidermal layers of the skin [30]. Passive diffusion of drugs from capillaries in the skin into perspiration seems to be the main pathway but excretion of substances via sebum and intercellular diffusion also contribute [2, 11]. The mechanism appears to be linked to the concentration gradient in which only the free fraction of drug unbound to proteins, diffuses through lipid membranes from plasma to sweat. Furthermore because under normal condition sweat with a mean pH of 6.3, is more acidic than blood, basic drugs tend to accumulate in sweat [31]. Excretion into sweat depends upon a drug's physical-chemical properties such as molecular mass, pKa, protein binding and lipophilicity. Therefore parent drugs that more easily cross membranes are expected to accumulate in sweat in greater concentrations than polar hydrophilic metabolites [11, 19, 32]. The passage of lipid-soluble compounds from blood to other fluids is also regulated by the pH of the matrices considered. A modified version of the Henderson-Hasselbalch equation, which uses the pKa and pH, allows theoretical calculation of the fluid-to-plasma concentration ratio [31]. There are other factors that appear not to have been considered for the transport of drugs into sweat. The rate at which drugs move from subcutaneous tissues to the skin surface could be significantly different from the rate at which drugs move from sweat glands to the skin surface. If the transit time for drug to move from subcutaneous tissue to sweat gland is considerably slower than time for drug to move from sweat gland to skin surface, clearance of the drug from the system would be significantly delayed. The

time to transport drug from adipose tissue to the skin surface could be relatively short or extremely long, it depends on the rate of transition between layers, the transport mechanism, the degree of reversibility and the magnitude of the equilibrium constants [25].

Cone E.J. [8] stated that the mechanism for drug entry into sweat was unclear, but most likely occurs by passive diffusion from blood to the sweat glands. An alternate mechanism could involve drug diffusion through the stratum corneum to the skin surface where drug would be dissolved in sweat. Skopp G. *et al.* [17] explained the passage of drug molecules from the skin capillaries into perspiration as a passive diffusion process governed by the same factors as the secretion into saliva. The elimination of a substances via sebum is delayed for many days as his the transcellular diffusion and transport by the keratinocytes. Additionally, drug binding to various skin fractions [33] and reabsorption of drugs from the skin have been observed [34]. Therefore, a continued presence of drugs on the skin surface results in the time period when blood or urine levels are already undetectable [35]. Skopp G. *et al.* [17] concluded that the material collected on the skin surface consists of various constituents and originates from various sources. The main analyte found on the skin surface is predominantly the parent drug. The time interval between drug consumption and detection on the skin surface depends on the nature of the particular drug and on the sensitivity of the analytical method used. In chronic abusers drug molecules are permanently present on the skin due to temporary reservoir of the stratum corneum [17].

4. ANALYTICAL METHODOLOGIES

The usefulness of a drug test resides in its ability to accurately detect the presence of parent drug or metabolites in biological fluids or tissues following human drug administration [20]. This definition reflects both chemical factors that influence test outcome such as sensitivity, specificity and accuracy, and pharmacologic consideration including dose, time and route of drug administration. Individual differences in rate of absorption, metabolism and excretion are also pharmacologic variables that may influence test outcome [20].

In the following section analytical methodologies used to detect drugs of abuse in sweat are reviewed; collection devices, immunochemical screening tests validated for this purpose and confirmatory analyses are also discussed.

a. Collection Devices

Two different approaches in testing for drugs in sweat can be performed. The first method is aimed to detect recent use of drugs (< 24 hours) and involves only collection of sweat at a point in time. It is mainly oriented to identify individuals who are under the influence of drugs. This kind of collection device (Drugwipe) will be discussed in the section of screening test. The second approach is based on patch technology and allows monitoring of illicit drug use for time windows wider than those provided by urine testing. This is because the patches can be worn for up to one week or even fourteen days. Drugs accumulate in the collection device, and little or no drug degradation seems to occur during this time interval. Systematic collection of sweat specimens is difficult because of unequal distribution of sweat glands on different parts of the body. Also there is irregular production of sweat volume which is highly dependent upon an individual's physical activity, emotional state, and the temperature of the environment [28].

Sweating maybe induced by exercise and several milliliters of sweat maybe collected in conjunction with an occlusive wrapping or gloves. Drugs maybe caused to diffuse into the skin under an electrical force but this procedure has not been employed as a sampling technique for diffusion of drugs out of the skin [16]. Small amounts of sweat maybe produced by electrical diffusion of pilocarpine into the skin or by warming the area; some devices have been developed using pilocarpine stimulation to increase sweat production [11, 16, 40].

Several commercial devices are available for the collection of sweat for drug analysis, however the most common application is via the sweat patch. In recent years extraordinary advances in analytical techniques have enabled the detection of drugs and drug metabolites in sweat. Early patch were made of absorbent cotton pads sandwiched between a waterproof, polyurethane, outer layer and a porous inner layer that is placed against the skin. A patch was later developed that included a chemical binding layer in the absorbent pad to prevent external water and other molecules from back diffusing into the absorptive pad [8].

Table **1** summarizes some important characteristics, such as the tipology of collection device, application site of the patch on human body, time of wearing, that are discussed in the following section.

In 1986 the use of a sample collection device (Macroduct) of human sweat for anion analysis was reported [41]. Cole DE. *et al.* [41] compared concentrations of chloride and sulfate in sweat obtained by use of the Macroduct capillary-coil collection device with results obtained by the conventional absorbent filter pad technique. Samples obtained with the device weighed less than those obtained conventionally, but sweat chloride concentrations were not significantly different. Background contamination, a problem with the filter pads, was negligible with the Macroduct collector [41]. Some paper [2, 42] refer about the use of the patch Band-aid that consists of an adhesive layer on a thin transparent film of surgical dressing to which a rectangular absorbent pad is attached. Water, oxygen, carbon dioxide and other gases pass freely through the polyurethane adhesive "Tegaderm" covering of the patch but molecules larger than vapor phase isopropanol are excluded by the molecular pore structure of the plastic membrane. An application of Band-aid type collection device [43] detected cocaine and its metabolites after intranasal assumption, indicating that the patch technology can be used to diagnose a single episode of cocaine use as far back as seven days. A few individuals developed slight redness and irritation from the patches which were apparent upon removal.

In 1996 a noninvasive and non-occlusive skin patch (Sudormed) was investigated for the systematic collection of drugs of abuse over a period of several days [44]. First, the applicability and user friendliness were tested by volunteers. A single dose experiment using theophylline as a model compound showed that there was a delay in time before the substance could be determined in the pad. The so-called sweat patch appears to be a valuable tool in clinical and forensic toxicology, as it offers a longer and prospective surveillance period compared with blood and urine testing [44]. In the same year benzodiazepines and metabolites [45], codeine and phenobarbital [46] were analyzed from sweat collected by means of Sudormed. Patches were removed at specified times over one week and drug content was determined by gas chromatography/mass spectrometry. Drugs were detectable in the 2-4 hours period following the administration.

Huestis M.A. *et al.* in 1999 [11] referred about the evaluation of two "Fastpatch" devices in a controlled clinical trial for the disposition of cocaine, codeine and their metabolites. These patches require only 30 minutes for sweat collection because they employ heat-induced sweat stimulation and a larger cellulose pad for increased drug collection. Through mild heating and a slightly large collection, "Fastpatch" [47] shows the promise of shorter required wear periods than other sweat patches, and possibly longer time periods of detected use. There were no significant differences in detection rates between 15, 20 and 30 minutes wear periods.

In 1990 a device called "PharmCheck" sweat patch [11] was marketed as a non occlusive sweat collection, consisting of a medical-grade cellulose blotted paper collection pad covered by a thin layer of polyurethane and acrylate adhesives. The absorption pad consists of inert cellulose that retains the non volatile components

of sweat collected from the surface of the skin. The release liner allows removal of the collection pad from the adhesive layer after patch use. Many advantages of the patch were observed, first of all it didn't alter the transport properties of the skin and water was not trapped against the skin minimizing the skin irritation. Moreover the patch is relatively impervious to environmental contamination [37] and appears to be relatively tamper-proof, in fact the patch adhesive is specially formulated so that it can only be applied once and cannot be removed and successfully reapplied to the skin surface [8]. The disadvantages include high inter-subject variability, the possibility of environmental contamination of the patch before the application or after removal, and the risk of accidental removal during a monitoring period.

The recommended procedure is to clean the skin with 70% isopropanol swabbing before application of the patch [48]. Each patch has unique nine-digit-number printed underneath the polyurethane layer that is visible through a window while that patch is being worn, useful in maintaining the chain of custody. The water component of sweat, vaporized by body heat, passes through the polyurethane; solids, salts and drugs excreted in the sweat or that pass through the skin are trapped on the collection pad. Although an other research [4] showed that drugs can remain on the skin for several days after application of the patch, the 70% isopropanol is not the most effective solvent in removal of drugs. Drugs deposited on the skin of drug free volunteers several days prior the application of the sweat patch were not completely removed by normal hygiene or the cleaning procedures recommended before application of the sweat patch [13]. The key component of the "PharmCheck" sweat patch, the membrane, has been tested for the passage of externally applied materials. Drugs in the uncharged state rapidly penetrated the membrane, but charged species were greatly slowed [13]. In basic media detectable concentrations of cocaine, methamphetamine and heroin were observed at the earliest collection time after drugs were placed on the outside of the membrane.

In conclusion numerous devices have been developed for collection of sweat specimens. The most common device in current use is the "PharmCheck" sweat patch which usually is worn by an individual for five to ten days. This device has been utilized in several field trials comparing sweat test results to conventional urinalysis and the results have been favorable.

b. Time Window

It was not established the optimal time of wearing sweat patches, although many scientists performed many studies for different illicit drugs. In 1998 Joseph R.E. *et al.* [36] established that after dosing some illicit drugs appeared in sebum within 1-2 hours and were detected for 1-2 days. A study [37] examining minimum length of wear necessary to detect recent or concurrent cocaine use in a convenience sample of active cocaine users established that the minimum duration that patches must be worn to detect recent or concurrent cocaine use is more than two hours and less than or equal to one day. Analyte concentrations increase significantly with increasing lengths of wear. The relative detection time of sweat respect to other specimens was suggested by Caplan Y.H. *et al.* [14]. Sweat provides a cumulative measure of drug use and could be applied to the monitoring of individuals in drug rehabilitation programs because it provides a prospective, rather than retrospective approach.

Time window depends in part on drug use pattern [38]; in fact three different patterns (chronic, occasional and no-use) are readily identified by daily urinalysis, while patch identifies only some of the occasional cocaine use episodes and virtually all of the frequent chronic users. Some studies [31, 39] suggest that there is a time dependent loss of drug during patch wearing over time. One of the most likely mechanism involved in drug loss is re-absorption back into the skin. Results on 3,4-methylenedioxymethamphetamine (MDMA) in sweat [39] showed an inflection in the kinetic at ten hours post-administration. This observation revealed the possibility that MDMA already incorporated in patches could be reabsorbed by the skin. According to this notice, the re-absorption (back transfer), degradation or hydrolysis, and loss of cocaine to the environment that may account for substantial loss of cocaine from skin sweat collection patches during patch wear, was studied [31].

c. Screening Tests

Sweat patch analysis requires extraction and sensitive chromatographic methods in combination with mass spectrometry to achieve an effective limit of quantification. Even though, immunoassays commonly used to screen samples prior to confirmation by gas chromatography/mass spectrometry (GC/MS), and mainly commercialized for urine samples, were also applied to alternative specimens such as sweat (Table **1**).

The first immunochemical detection of drugs in apocrine sweat collected the samples from the axillary perspiration [49]. The determination of the drugs (sum of parent drug and metabolites) was performed by radioimmunoassay (RIA). Measurable drug concentrations of cannabinoids, benzodiazepines, cocaine, barbiturates, morphine, methadone and cotinine were found in all samples. Balabanova S. *et al.* [40] investigated the presence of cocaine, morphine and methadone in sweat samples obtained after stimulation of the eccrine sweat glands.

A different system [42] for the analysis of cocaine in sweat employed a solid-phase enzyme immunoassay (EIA) involving modified microtiter plates after extraction of pad with acetate buffer and methanol; this procedure showed to have cross-reactivity for cocaine and benzoylecgonine with higher concentration of parent drug.

In 2001 [50] two types of immunoassays (RIA and microplate enzyme immunoassay), were compared to detect and quantitate cocaine, heroin and metabolites in extracts of sweat patches. Assays were first evaluated for sensitivity in detection of the different concentrations of analytes known to be excreted in sweat. Various cross-reactivities were evaluated for both devices. In 2004 Moody D.E.*et al.* [51] reported a comparative analysis of sweat patches for cocaine and metabolites by RIA and gas chromatography-positive ion chemical ionization mass spectrometry. Sweat patches worn by subjects receiving treatment for cocaine dependence to compare the procedures were analyzed. Patches were extracted with acetate buffer pH5 directly analyzed. Time expended on performing RIA analysis of all the samples was cost-effective when the results were used to exclude negatives from and predict dilutions required for GC-MS analysis. RIA offers a sensitive and specific alternative initial test for cocaine determination in extracts of sweat patches [51].

Although the initial research in the area of alternative specimens utilized RIA, newer non isotopic commercial immunoassays are widely available for screening of drugs and drug metabolites. Techniques such as Enzyme-Linked Immunosorbent Assay (ELISA), have been adapted for detection of analytes in sweat patches [14]. Kidwell D.A. *et al.* [38] compared three different immunoassays to screen specimens for cocaine on matrices not commonly tested: a modified manual Microgenics CEDIA, Cozart ELISA and OraSure ELISA. Before the immunochemical test dried skin swabs or patches were extracted with two portion of 0,1M of chloride acid. Both the Cozart and OraSure cocaine immunoassays performed similarly and showed a reasonably strong correlation with each other. In contrast, although the modified Microgenics assay showed the requisite sensitivity for the matrices examined, it had poor precision when run in a manual mode.

A rapid onsite test called "DrugWipe" immunochemical strip test, was also assessed; the device is a pen size, immunochemical-

based test strip used for the detection of drugs of abuse on surface. The wiping part enables the user to sample drug particles from any kind of surface such as the skin; it is simple to use and results can be obtained after two minutes. In 1999 Mura P. *et al.* [52] evaluated the results of tests when applied to sweat. Regular users of cannabis and persons who denied consuming it were studied. The results obtained with DrugWipe in sweat were compared with anamnesis data. The Authors observed that DrugWipe may be useful for screening cannabis in sweat when the intake took place less than two hours before. Potential drug users participated voluntarily in a study [53] to evaluate the usefulness of the DrugWipe for the screening of cocaine, opiates, amphetamine and cannabinoids referring about advantages and disadvantages of the test. DrugWipe for the analyses of drugs of abuse in sweat [54] have also been applied to healthy volunteers familiar with the effects of MDMA after single oral dose. MDMA consumption could be detected at two hours and for as long as twelve hours after drug administration. Pichini S. *et al.* [39] found the onsite test positive at 1.5 hours but few false-negative results appeared in the first six hours after administration.

A particular application [55] regarding an immunoassay based technique was recently used for the detection of psychoactive substances in the sweat deposited within fingermarks of a narcotic drug user using white light and/or a fluorescence light source. In particular Hazarika P. *et al.* [55] showed that morphine can be detected in the sweat deposited within a latent fingermark, concluding that fingermarks images can provide information on drug usage of an individual.

d. Confirmatory Analytical Techniques

Immunochemical screening always needs confirmatory analysis to be performed by chromatographic techniques. When alternative matrices are used the employ of confirmatory analytical techniques is mandatory. Advances in sensitive methodologies have enabled the analysis of drugs in unconventional biological materials such as sweat. Scientific literature refers about the detection of drugs of abuse in sweat employing both gas and liquid chromatographic methods.

Many papers were published about the detection of xenobiotics in sweat (Table **1**) by gas chromatography mainly coupled with mass spectrometry [11, 12, 25, 27, 30-32, 36, 39, 42, 45-48, 51, 53, 56-74]. Some of them discuss sweat combined with other biological matrices. In this section we discuss some representative papers referring about the analytical procedure applied to many compounds. Kintz P. *et al.* [12] in 1997 conducted a study on sweat patches applied to some subjects during heroin maintenance program. The target drugs were extracted in acetonitrile solution and the residues were analyzed by gas chromatography-mass spectrometry in electron impact mode directly and after silylation. Heroin was the major drug present in sweat, followed by 6-acetylmorphine and morphine. No correlation between the doses of heroin administered and the concentrations of heroin measured in sweat, was observed. Sweat testing for cocaine, codeine and metabolites by EIA and GC/MS was performed [11] on voluntary people administered with cocaine and codeine. Sweat patches were eluted by sodium acetate buffer successively extracted by Solid Phase Extraction (SPE) and derivatized to obtain silyl-derivatives analyzed by GC/MS in selected ion monitoring mode. The authors concluded that the combination of EIA and GC/MS analysis was sensitive enough to detect cocaine in sweat after minimal abuse. More recently a sensitive gas chromatography-negative ion chemical ionization mass spectrometry (GC/MS-NICI) method [65] was developed and validated for the measurement of Delta(9)-tetrahydrocannabinol (THC) in human sweat patches. Patches were extracted with methanol-sodium acetate buffer pH 5.0 for 30 minutes. Extracted solution was diluted with sodium acetate buffer (pH 4.5) and extracted by solid-phase extraction columns (CleanScreen; United Chemical Technologies). Dried extracts were derivatized with trifluoroacetic acid and analyzed by gas chromatograph interfaced with an mass selective detector operated in NICI-selected ion-monitoring mode. The same paper studied various potential interfering compounds added to low quality-control samples founding to not influence THC quantification. Saito T. *et al.* [65] stated that GC/MS-NICI assay for THC in human sweat provides adequate sensitivity and performance characteristics for analyzing THC in sweat patches and meets the requirements of the proposed Substance Abuse and Mental Health Services Administration's guidelines (SAMHSA) for sweat testing [6].

A semi-quantitative gas chromatographic/mass spectrometric method [66] was developed to simultaneously detect cocaine and cocaethylene in sweat samples collected by PharmChek, eluted with sodium acetate buffer (pH 5.0) and extracted by solid-phase microextraction (SPME). The method showed to be very simple, rapid and sensitive. A positive chemical ionization gas chromatography/mass spectrometric method was validated to simultaneously quantify drugs and metabolites in skin collected after controlled administration of methamphetamine, cocaine and codeine [68]. Amphetamines were eluted from PharmChek sweat patches [70] with sodium acetate buffer, extracted with disk solid phase extraction and analyzed using GC/MS-Electron Impact mode with a fully validated procedure that permitted the simultaneous analysis of multiple amphetamine analogs in human sweat. Another application for amphetamine detection in sweat patches following controlled MDMA administration was performed by Barnes AJ. *et al.* [73]. A sensitive, specific and validated GC/MS method (electron impact ionization and selected ion monitoring) was presented to simultaneously quantify methadone, heroin, cocaine and metabolites in sweat [71].

The successful interface of liquid chromatography with mass spectrometry (LC-MS) has brought a new light into bioanalytical and forensic sciences as it allows the detection of drugs and metabolites at concentrations that are difficult to analyze using the more commonly adopted GC/MS based techniques [75-83]. Liquid chromatography allows the separation of enantiomeric mixtures of xenobiotics employing chiral stationary phases [80]. In 1998 [80] an enantioselective separation of methadone was obtained using an alpha-1-acid glycoprotein column and liquid chromatography/Ion Spray Mass Spectrometry. The separation of R- and S-methadone can be used to document some physiologic mechanisms of excretion and incorporation into sweat. The combination of LC-MS with innovative instrumentation such as triple quadrupoles, ion traps and time-of-flight mass spectrometers has been focused [82]. Methyl phenidate and ritanilic acid [76], buprenorphine, methadone, cocaine, heroin, nicotine and their main metabolites [75] were determined in patches using previously validated liquid chromatography-electrospray ionization mass spectrometric methods. A procedure based on LC-MS/MS was described [83] for the determination of atomoxetine and its metabolites in sweat and other matrices. Analytes were extracted from sweat patch with tert-butyl methyl ether and the organic layer was evaporated and redissolved in mobile phase. Separated analytes were identified and quantified by positive electrospray ionization tandem mass spectrometry and in multiple reaction monitoring acquisition mode. Kintz P. *et al.* [84] applied sweat patches to known heroin abusers coming from a detoxification center; target drugs (opiates, cocaine, cannabinoids, benzodiazepines, amphetamines and buprenorphine) were analyzed either by GC/MS or LC-MS depending on the target compound. de Oliveira C.D.R. *et al.* [5] published a review of chromatographic procedures for determination of amphetamines, cannabinoids, opiates, nicotine, cocaine and alcohol in alternative biological matrices. Gas chromatographic and liquid chromatographic procedures with different detectors and sample preparation techniques such as liquid/liquid, SPE and SPME extraction were discussed.

5. SWEAT VERSUS OTHER MATRICES

The selection of the specimens for drugs analysis is influenced by a variety of factors, principally ease of specimens collection, analytical and testing considerations and interpretation of results. Moreover each specimen gives different information respect to the detection window. Many papers simultaneously studied different biological matrices, including sweat, for the detection of drugs of abuse, but some of them focused on the direct comparison between the different specimens. The new matrices demonstrate some distinct advantages over urinalysis, e.g. less invasive procedures, different time course of drug detection [18]. A comparison [9] between urine, sweat and hair was performed to identify the best matrix in drug testing. In contrast with urine, hair analysis has a wide window of detection, ranging from months to years. Testing individuals for illicit drugs with sweat patches worn continually would provide effective coverage for a week.

Smith FB. *et al.* [3] compared the concentrations of cocaine and benzoylecgonine in the hair, saliva, skin secretions and urine samples of cocaine – using mothers, their children, and other adults living in the same environment. Most of the skin swabs from the adult subjects and most of their children were positive for cocaine. The presence of cocaine in swabs could indicate that it originated from sweat. However negative child urine and saliva results contradict this hypothesis and imply recent surface contact.

Joseph R.E. *et al.* [36] examining the disposition of cocaine, codeine, and metabolites in stratum corneum, sebum and plasma collected from African-American males after administrations of cocaine and codeine compared the results with the distribution in sweat. Stratum corneum consists of 15-20 layers of keratinized cells that are similar to hair with the exception that fewer disulfide bonds are present in stratum corneum compared with hair. Sebum is an oily substance composed primarily of wax ester fatty acids produced in cells of sebaceous glands. No relationship was observed between drug concentrations in sebum and stratum corneum compared with dose. Interpretation of drug distribution and elimination in sebum and stratum corneum was complicated by possible contamination of specimens with drugs from sweat.

The analysis of urine and patch test for methadone, opiates and cocaine metabolites was performed on patients with a diagnosis of opiate addiction prescribed methadone [85]. There was good agreement between sweat patches and urine tests for methadone and opiates, but only moderate agreement for benzoylecgonine probably due to urine tests only detecting use over the last 2-3- days. Sweat patches may be more sensitive and detecting illicit drug use as they provide a longer period of collection. A comparison between urine and sweat patches results obtained from a woman with a history of chronic methamphetamine and cocaine abuse [86] was reported. The results of the study rise further questions about the preferential use of the sweat patch in detecting new episodes of drug use in formerly chronic drug users. Advances analytical techniques have enabled the detection of drugs in alternative biological specimens for the purposes of workplace testing. Caplan YH. *et al.* [14] evaluated some of these specimens (oral fluid, hair and sweat) in order to determine their utility in Federally Regulated programs focusing the attention on advantages and disadvantages of the matrices considered.

To compare the efficacy of sweat testing versus urine testing for detecting drug use [62] paired sweat patches were applied and removed quickly and compared to 3-5 consecutive urine specimens from patients in a methadone maintenance treatment program. The identification of heroin and / or 6-acetylmorphine in sweat patches confirmed the use of heroin in 78% of the positive cases and differentiated illicit heroin use from possible ingestion of codeine or opiate-containing foods.

Kidwell DA. *et al.* [38] highlighted advantages and disadvantages of daily urine and sweat patches to establish the pattern of the drug use among cocaine abusers. The patch identified some of the occasional cocaine use episodes and all of the frequent chronic users. A comparison [72] between hair and sweat was performed on heroin abusers in methadone treatment calculating the ratio between methadone and its metabolite in both biological matrices; these ratio appeared to be comparable.

In conclusion each biological matrix shows peculiarities that could be used in different context hence the role of the forensic toxicologist is also the choice of the most suitable sample. Sweat samples provide cumulative measure of drug exposure, they are able to monitor drug intake for a period of days to weeks, they detect parent drugs and metabolites, the collection is non invasive and the devices are relatively tamper proof. However the large variation in sweat production, the volume unknown and the high Intersubject variability are some of the major disadvantages.

6. MONOGRAPHIC STUDIES

In this section we summarized papers regarding the most common substances of abuse in sweat found in the international literature.

a. Amphetamines

Amphetamines are powerful psychostimulants, producing increased alertness, wakefulness, insomnia, energy and self-confidence in association with decreased fatigue and appetite as well as enhanced mood, well-being and euphoria [87]. Hepatic metabolism is extensive in most cases, but a significant percentage of the drug always remains unaltered. Amphetamine and related compounds are weak bases with a relatively low molecular weight [87]. These characteristics allow amphetamine-type stimulants to diffuse easily across cell membranes and lipid layers and to those tissues or biological substrates with a more acidic pH than blood, facilitating their detection in alternative matrices [87].

Already in the years ’80 a method for the detection of methamphetamine and its major metabolite in sweat from habitual users by mass fragmentography has been developed [88]. Sweat samples were extracted with methanol and after trifluoroacetyl derivatization, analyzed by mass fragmentography. Later in 1996 [57] sweat was collected with the “PharmChek TM” sweat patch and drugs were eluted from the collection pad of the patch. A solid phase, enzyme immunoassay using microtiter plates was modified for analysis of methamphetamine. The results were confirmed by GC/MS. Barnes A.J. *et al*. [89] confirmed these results studying the excretion of MAMP and AMP after controlled MAMP administration. A procedure based on GC/MS [90] for the simultaneous identification of N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB) and its desmethylated metabolite 3,4-(methylene dioxyphenyl)-2-butanamine (BDB) in sweat specimens were presented. Sweat specimens, which were collected by a sweat patch, were tested after methanolic elution. MBDB was present in higher concentrations than its metabolite. A review [91] of the procedures for the determination of MDMA derivatives, methylendioxyamphetamine (MDA), MDMA, methylendioxyethylamphetamine (MDEA), MBDB in saliva, sweat and hair was reported. The parent drug was found to be always in higher concentrations than metabolites. The development and validation of a method for the simultaneous quantification of some amphetamines related drugs in sweat was also reported [70].

A brief review [92] for the detection of AMP and methylendioxy-derivatives in sweat was reported. According to guidelines for drug monitoring using sweat as alternative specimens proposed by SAMHSA requirements for a positive sweat test include amphetamines screen test with higher than 25 nanograms/patch and a con-

firmation cut off of 25 nanograms/patch for amphetamines and methylenedioxy-derivatives. De Martinis BS [92] reviewed the indexed literature founding limits of detection and quantification ranging from 0.72 ng/patch to 5 ng/patch and from 1.4 ng/patch to 5 ng/patch for all analytes respectively.

Pharmacokinetic studies [81, 39] were performed after the oral administration of MDMA to healthy volunteers known to be recreational MDMA-users. Sweat wipes were collected after administration and the MDMA levels averaged 25 nanograms/wipe [81], also demonstrating large intersubject variability with peak MDMA concentrations for the same dose varied in magnitude 30-fold [39]. Recently the disposition of MDMA and metabolites in human sweat following controlled MDMA administration was also reported by Barnes A.J. *et al.* [73]. MDMA was the primary analyte detected with concentrations up to 3007 nanograms/patch. MDA was detected at much lower concentrations, whereas no HMMA or HMA was detected. The variability in sweat excretion suggests that results should be interpreted qualitatively rather than quantitatively.

b. Cannabis

The use of marijuana and hashish (derived from cannabis Sativa) mostly by smoking, produces sedation, euphoria, hallucinations or temporal distortion. The main psychoactive compound is THC which is first biotransformed to an active metabolite, 11-hidroxy-THC which in turn is rapidly converted to an inactive metabolite, 11-nor-9-carboxy-THC [8]. In contrast to the majority of drugs of abuse which are weak base and tend to concentrate in biological matrices more acidic than plasma, THC is a neutral molecule and its diffusion is expected to be slower [93]. Not surprisingly lipophilic drugs can be detected in skin and adipose tissue. Johansson E. [94] demonstrated that in heavy marijuana users, THC remained in adipose tissue up to 28 days after smoking. Only a few papers were found in literature referring about the detection of cannabis in sweat. Early paper detecting THC in sweat [49] found the parent drug to be the primary analyte. In a study conducted in a detoxification center, sweat patches were applied to 20 known heroin abusers. Target drugs analyzed either by GC-MS or LC-MS included delta9-tetrahydrocannabinol, identified in nine cases (4-38 ng/patch) [84]. In 1999 Staub C. [95] found THC in low amount and the principal acidic urinary metabolite (11-nor-9-carboxy-THC) has been never detected in sweat. An editorial discussion on the usefulness of sweat testing of the detection of cannabis smoke has been reported in 2004 [93]. In order to demonstrate an intake of cannabis Mura P. *et al.* [52] evaluated the results of DrugWipe. Regular users of cannabis and persons who denied consuming it were studied. The results obtained were compared with anamnesis data and indicated that DrugWipe could be useful for screening cannabis in sweat when the intake took place less than two hours before. The non-instrumental immunoassay Drugwipe was also used in a Belgian study for the screening of cocaine, opiates, amphetamine and cannabinoids in saliva and sweat [53]. Procedures using GC/MS to test for THC in forehead wipes were developed and validated [96, 65]. Injured drivers were tested, some of them were positive for THC, but metabolites were never detected [96]. Saito T. *et al.* [65] developed an assay with adequate sensitivity and performance characteristics for analyzing THC in sweat patches meeting the requirements of the proposed SAMHSA's guidelines for sweat testing [6]. Huestis MA. *et al.* [97] evaluated THC excretion in daily cannabis users after cessation of drug use. Moreover some subjects were administered oral doses of THC for five consecutive days; the results demonstrated that THC does not readily enter sweat following oral ingestion.

c. Cocaine

Cocaine is a powerful addictive stimulant drug extracted from "Erythroxylon coca" leaves that can be administered intranasally, or by intravenous or intramuscular injection. It can also be taken orally, sublingually, vaginally or rectally or it can be smoked. The human metabolism transforms cocaine into two major metabolites (benzoylecgonine and ecgonine methylester) and some other minor metabolites [8]. In recent years cocaine is becoming the most popular drug of abuse and many studies on its detection in sweat have been published.

A clinical study [43] examined a skin patch method of monitoring drug use after two different treatment doses. Analysis of the patch content yielded cocaine levels from the cocaine subjects that accurately reflected usage. Mean levels were significantly different for the two treatment doses. The data do indicate that the patch technology can be used to diagnose a single episode of cocaine use as far back as seven days.

The use of a sweat patch for detecting cocaine abuse in cocaine-dependent patients participating in a clinical trial was studied [98]. The reliability and validity of quantitative sweat patch results, the possible degradation of cocaine to benzoylecgonine as a function of the length of time that a patch is worn and the relative costs associated with sweat patch were also evaluated. The results revealed no significant degradation of cocaine to benzoylecgonine associated with wearing the patch. The excretion of cocaine in sweat of volunteers receiving low and high doses, was again evaluated in 2005 [30]. Pharm-Chek sweat patches were collected before the administration, during and after controlled dose. Cocaine was the primary analyte detected and frequently the only one.

Some contamination studies were performed on different population. An application of sweat test was performed on cocaine-using mothers and their children [3]. To distinguish actual drug use from passive exposure to the drug a comparison between forehead swabs and other biological materials were performed. In a second trial a random population of adults at a major United States University [4] was studied. Sweat was obtained by wiping the forehead with a cosmetic puff containing isopropanol. Moderate amounts of cocaine and benzoylecgonine are slowly lost when placed on the skin possibly due to absorption. To test the persistence of larger amounts of cocaine and benzoylecgonine on the skin through removal by normal hygiene and absorption by the body, two sets of experiments were carried out. After three days of normal hygiene and three removal steps the drugs were undetectable.

More recently Kidwell D.A. *et al.* [38] made a comparison of daily urine, sweat and skin swabs among cocaine users. Large quantities of cocaine were found on the skin of individuals with urine positivity and an evaluation of drug contamination on the external patch membrane was performed. Immunoassays were studied and validated in various papers using different techniques. A solid-phase enzyme immunoassay [42] involving microtiter plates was modified for the analysis of cocaine in sweat, collected with the PharmChek patch that contained primarily parent cocaine, and the method was validated for qualitative detection.

The monitoring of cocaine use was performed in substance-abuse-treatment patients by sweat testing [61]. Sweat and urine specimens were collected from methadone-maintenance patients to evaluate the use of sweat testing to monitor cocaine use through ELISA test. Immunoassays were also evaluated by Moody D.E. *et al.* [50] for their ability in the detection of cocaine and metabolites discussing the cross reactivity. Later a RIA method [51] using sweat patches worn by subjects receiving treatment for cocaine dependence was developed.

Various methodologies were proposed to identify and quantitate cocaine and its metabolites. A semi-quantitative method (SPME followed by GC/MS) was elaborated to simultaneously detect cocaine and cocaethylene in sweat [66]. GC/MS [68, 71] and LC-MS methods [75] for many substances including cocaine and its metabolites in sweat were developed and comprehensively validated. These methods permit fast and simultaneous quantification of many

drugs and metabolites in sweat patches, with good selectivity and sensitivity.

The minimum length of wear necessary to detect recent or concurrent cocaine use in a convenience sample of active cocaine users was examined [37]. Differences in analyte concentrations with increasing longer-term wear were observed. Some studies compared different sites of application of the patches to establish the best collection point. Huestis M.A. *et al.* [11] reviewed sweat testing for cocaine discussing that diversifying the site of collection change drug disposition in sweat. Generally concentration of cocaine are higher in sweat specimens collected on the hand respect to the torso. This observation is likely to be due to differences in the anatomy and physiology of the skin on the palm of the hand compared to the torso skin. Uemura N. *et al.* in 2004 [31] also investigated the effect of sweat patch location (back and shoulder) on cocaine levels after controlled intravenous cocaine exposure in different subjects. The analysis showed cocaine and metabolites levels in sweat eightfold higher on the back than those on the shoulders.

A continuing social problem is presented by the large number of individuals who smoke crack; crack is a mixture of cocaine hydrochloride and sodium hydrogen carbonate which liberates the cocaine base from the hydrochloride with some cracking noise. Liberty HJ *et al.* [47] have identified unique pyrolysis products of crack or burned cocaine as anhydroecgonine methylester and ecgonidine through GC/MS that allow for the detection of crack use distinct from other cocaine use.

d. Opiates

Opium is a natural product containing morphine as the principal alkaloid. Illicit market synthesizes heroin adding two acetyl groups to morphine in order to obtain a stronger drug. Following intake, heroin is rapidly deacetylated to 6-acetylmorphine which is then further hydrolyzed to morphine at a slower rate [8].

Many papers refer that opiates are excreted in sweat, and the parent drug is the predominate analyte found. Heroin and its metabolites [2] were investigated in sweat patches in order to evaluate the possible use of alternative matrices. The data suggested that sweat patches could serve as a useful monitoring device in surveillance of individuals in treatment and probation programs. In a study conducted in a detoxification center [84], sweat patches were applied to known heroin abusers, to detect heroin, 6-acetylmorphine, morphine and codeine. When detected, heroin was always present in lower concentrations than 6-acetylmorphine, which was the major analyte found in sweat. It is noteworthy that sweat is one of the few matrices in which heroin is readily detected. After opiate (codeine, or heroin or poppy seeds) administration [48] patch performances were evaluated. Heroin and 6-acetylmorphine or codeine but little morphine were found in sweat after heroin or codeine administration as contrasted to the metabolite profile found in urine or blood. Heroin and 6-acetylmorphine or codeine appear in sweat within 24 hours of administration of opiates in controlled studies and peak within the first three days. An other study [12] conducted during an heroin maintenance program applied sweat patches to subjects that received intravenously two or three doses of heroin hydrochloride ranging from 80 to 1000 mg/day. The sweat patch was applied ten minutes before the first dosage and removed approximately 24 hours later, minutes before the next dosage. Except in one case, heroin was the major drug present in sweat, followed by 6-acetylmorphine and morphine. No correlation between the doses of heroin administered and the concentrations of heroin measured in sweat were observed.

The time course, the cumulative excretion, the intra-subject variability, the influence of site application, and the concentrations of codeine in sweat following administration of a single dose of the drug, was also performed [46]. Codeine was detectable at one hour following the administration, and a plateau concentration was observed on the third day. The peak codeine concentration was determined during the 12-24 hours period. Morphine was never detected in sweat and inter-subject variability was enormous, but Kintz P. *et al.* [46] suggest that the sweat patch technology can be useful for documenting drug use over a one week period of surveillance. Codeine phosphate was orally administered to six subjects testing sweat immediately with the Drugwipe and applying the sweat patch at the same time [99]. Codeine was quantified in the patch by GC/MS. In all subjects except one, the Drugwipe tested positive for opiates. After controlled oral codeine administration [32, 68] significant variability in concentrations was observed in patches applied to various locations in the upper body. Codeine was detected within one hour and peaked within 24 hours and no metabolites were detected. The study comprehensively evaluated hourly and weekly sweat patches to characterize the duration, accumulation, reproducibility, time of first appearance and dose concentration relationship of codeine excretion in sweat.

Methadone is a opioid pain reliever, similar to morphine. It reduces withdrawal symptoms in people addicted to heroin or other narcotic drugs without causing the "high" associated with the drug addiction. It is used in detoxification and maintenance programs for the management of physical dependence of narcotics [1]. Some studies were found in literature about its possible detection on sweat samples. Henderson G.L. *et al.* [56] already in 1973 referred about the excretion of methadone and metabolites in human sweat. Later [44, 80, 85, 100] the presence of methadone was again investigated in 24 hours perspiration samples obtained from patients receiving daily maintenance doses of the drug. No correlation between the dose methadone administered and the concentrations of methadone in sweat was observed but sweat patches were reliable giving valid results for patient on maintenance methadone.

The authors of the present review didn't find papers referring the detection of methadone in sweat in the scientific literature in the period 1998 – 2007. Successively [72] a comparison between hair and sweat samples from patients in long term maintenance therapy was performed. Fucci N. *et al.* [72] referred about their experience with sweat applied to supervise methadone therapy of heroin abusers. Some advantages respect to hair were found such the time window of sweat shorter than hair that allows the doctor easily to check the therapeutic program of abusers. A good agreement between patients, when the application of the patch was proposed instead of the daily collection of urine or hair cut, was underlined. The development of an analytical method for the simultaneous quantification of methadone and other xenobiotics in sweat is reported by Brunet B.R. *et al.* [71]. The excretion of methadone in sweat of pregnant women after controlled methadone administration was also studied [74]. Methadone was present in all weekly patches, correlation between patch concentrations and total amount of drug administered and concentrations and duration of patch wear were both weak.

Buprenorphine is a strong opioid painkiller which is used to treat moderate to severe pain; it is currently under investigation as a pharmacotherapy to treat abusers for opioid dependence [1]. Some papers [26, 101] evaluated the utility of sweat testing for monitoring of drug use in outpatient clinical settings and opioid dependent pregnant women. Chawarski M.C. *et al.* [26] compared sweat toxicology with urine toxicology and self report drug use during a randomized clinical trial of the efficacy of buprenorphine for treatment of opioid dependence in primary care settings. The other research [101] evaluated buprenorphine and its metabolites pharmacokinetics after sublingual administration to pregnant women, suggesting that, like methadone, upward dose adjustments may be needed with advancing gestation.

e. Other Substances

Other substances were investigated in human sweat, such as nicotine, the object of some scientific studies before the year 2000.

In 1990 [49] specimens of apocrine and eccrine sweat collected without and after pilocarpine stimulation from smokers and non-smokers exposed to tobacco smoke were investigated. The concentrations were determined by RIA, so that the values obtained represent the concentrations of nicotine plus its metabolites, e.g. cotinine. The levels measured in apocrine sweat were higher than those in eccrine sweat. The presence of nicotine in sweat obtained from smokers and non-smoker exposed to tobacco smoke after four hours to eight days of nicotine-free time was investigated [102]. The sum of nicotine and its metabolites were determined by RIA. The presence of unchanged nicotine was revealed by GC/MS. In a study conducted with cigarettes smokers and nonsmokers [60], Pharm-Chek sweat patches were applied to subjects for 72 hours. Nicotine was determined using GC/MS, and it was not detected in non-exposed nonsmokers, while it was found in passive and active smokers.

Not only narcotics and stimulants, but also many alkaloids and barbiturates are excreted in the sweat and detected quantitatively by the same principles [46, 103]. To determine the time course, the cumulative excretion, the intra-subject variability, the influence of site application, and the concentrations of phenobarbital in sweat following administration of a single dose of the drug a clinical study was performed [46]. Phenobarbital was first observed three hours after administration, and cumulative excretion was continual throughout the week. Intersubject variability was enormous with the concentrations in the range of 0.5 - 33 nanograms/patch. These data suggest that the sweat patch technology can be useful for documenting drug use over a one-week period of surveillance.

Benzodiazepines were also studied [45] to determine the cumulative excretion, the time course, the dose-concentration relationship, and concentrations of diazepam and its metabolites in sweat following oral administration of single dose of the drug. Irrespective of the time of collection, diazepam and nordiazepam were present, but oxazepam was never detected. Drugs were detectable in the two to four hours period following the administration. Concentrations were in the range 0.1 to 6.0 nanograms/patch for both drugs.

The determination of clozapine in sweat was performed using a liquid chromatographic method [77]. The correlation between clozapine levels in hair and sweat and the daily dose was also studied. The main active ingredient of the hallucinogenic mint Salvia Divinorum (Salvinorin A) was also investigated in sweat but never detected from consumers [67]. After controlled administration of gamma-hydroxybutyric acid (GHB) sweat and other biological fluids were analyzed by GC/MS [69]. GHB was detected in sweat at low concentrations, hence this biological matrix appears not to be suitable for monitoring GHB consumption. Methylphenidate, a prescription amphetamine derivative used in the treatment of attention-deficit hyperactivity disorder, has been amply described in conventional biological matrices. Recently, the excretion of methylphenidate and its principal metabolite (ritalinic acid) in sweat has been studied [76, 104]. Atomoxetine, a drug approved for the treatment of attention–deficit hyperactivity disorder, was recently detected by a LC-MS/MS procedure in conventional and non-conventional biological matrices from individuals in drug treatment [83]. Sunitinib, used to treat tumors such as gastrointestinal, renal cell carcinoma, pancreatic neuroendocrine tumors, was detected in sweat collected from a patient therapeutically treated with the drug [105].

7. FORENSIC APPLICATIONS

Sweat testing received particular attention by scientists for its possible application on roadside and workplace drug testing that would be the object of the following sections.

a. Roadside Drug Testing

To establish driver's impairment sweat samples are obtained by wiping the forehead. Some studies were found in literature referring experiences in this context.

An interesting European study called "ROSITA" (ROadSIde Testing Assessment) was born to evaluate devices for the analysis of sweat and other matrices in order to control drivers under the influence of drugs (www.rosita.org). The objective of the ROSITA study is *"to identify the requirements for road side testing equipment, and to make an international comparative assessment of existing equipment or prototypes. The assessment will address road side testing result validity, equipment reliability, usability and usage costs"*.

The first results published [106, 107] referred about the evaluation of various analytical devices to test sweat. Eight nations were enrolled and about 3000 drivers were tested to evaluate the role played by the drugs of abuse in the drive performances. The usefulness of sweat was demonstrated, it was best accepted respect to other matrices by drivers, but the need to more investigation was underlined [17].

Prospective analytical studies [96, 108] were performed in large population of drivers implicated in non-fatal traffic accidents to determine the significance of drug levels observed in blood, urine, saliva and sweat. The samples were tested for pharmaceuticals and drugs of abuse by hyphenated chromatographic methods. The authors observed that a limitation in the use of the sweat for roadside testing is the absence of a suitable immunoassay to detect the parent compound. Sweat samples by wiping the forehead were obtained from drivers who failed the field sobriety tests at police roadblocks [64]. The positive predictive value of sweat wipe analysis by GC/MS was over 90% for cocaine and amphetamines and 80% for cannabis.

A global overview [109] on the issue of drugs and driving discussing the utility of alternative specimens including sweat was presented by Walsh J.M. *et al.* in the year 2004. A special attention for the effects of medicinal and illegal drugs on driving performance was reported and Walsh J.M. *et al.* drew conclusion regarding the risk of the drug to traffic safety.

b. Workplace Drug Testing

Workplace drug testing is a well-established application of forensic toxicology and it aims to reduce workplace accidents caused by affected workers. Several classes of abused substances may be involved, such as alcohol, amphetamines, cannabis, cocaine, opiates and also prescription drugs, such as benzodiazepines. Since the 1970's, urine drug testing has been the most common technique for detecting drug use in the workplace.

National laws of each country provide the underpinnings of drug-testing programs, but most countries have not addressed use of these alternate matrices. In 2001 [18] Cone EJ. reviewed national and local laws of many countries providing the employ of drug-testing programs, discussing that only a few countries have statutes that specifically mention the use of alternate biological matrices. In our knowledge only very few advances have been made in the last ten years. Caplan Y.H. *et al.* [14] discussed about the use of alternative specimens for workplace drug testing suggesting that oral fluid, hair and sweat appear to sufficiently meet the requirements to be added. No other paper were found in the recent literature. On 13th April 2004 the U.S. Department of Health and human services (www.samhsa.gov) published a notice in the Federal Register proposing to establish also scientific and technical guidelines for the testing of alternative matrices (like sweat) in addition to urine specimens [6].

c. Other Forensic Applications

Forensic toxicology can be involved in several situations to document impairment, such as: crime under influence, date rape, psychiatric disorders, determination of the cause of death. In some particular situations, it can be very cautious to investigate exposure to psycho-active drugs, due to late sampling of biological specimens.

In the context of the "crime under the influence" many biological specimens have been tested to verify the use of drugs after sexual assaults such as drugs spiked in food. Only a paper was found [63] exploiting the use of sweat to detect drugs administered during sexual assault.

The use of sweat in doping context was referred [59] to detect anabolic steroids, diuretics and corticosteroids. Two papers regarding the possible application of sweat in sport doping were published [110, 111] that discussed sampling, analytical procedures and interpretation of the results.

Being the skin constantly exposed to sweat and sebum, drugs may be sequestered in this tissue. Moreover there is evidence that after chronic exposure lipophilic drugs may be stored in adipose tissue creating a drug depot. Hence in this section we consider some paper describing the analysis of drugs in these matrices. Yang W. *et al.* [68] described the analysis of 55 skin biopsies collected from 15 volunteers after controlled administration of methamphetamine, cocaine and codeine. Levisky J.A. [25] considered the adipose tissue and skin in illicit drug related deaths for qualitative and quantitative analyses removing the tissues from the abdominal region during the autopsy.

Particular applications detected methamphetamine in garments belonging to known-abusers [78] extracting the drugs from the textile using a mixture of organic solvents and morphine in the sweat deposited within fingermarks of a narcotic drug user [55].

8. DRUG USE DURING PREGNANCY

The maternal abuse is often underestimated due to the stigma of drug use during pregnancy and the accompanying legal, ethical and economic issues. In utero drug exposure can have a severe impact not only on the development of the fetus, but also on the child during later stages of life. Accurate identification of in utero drug exposure has important implications for the care of the mother and child, but can raise difficult legal issues. Detection of in utero drug exposure has traditionally been accomplished through urine testing; however, the window of detection is short, reflecting drug use for only a few days before delivery, hence other biological matrices such as sweat can be used [112]. Maternal drug use during pregnancy can be monitored with alternative matrices such as sweat testing that offers a longer window of detection of about one week and decreases the likelihood of missing recent use.

Some reviews regarding various biological matrices [113] and bioanalytical methods [114] useful for the detection of exposure from different gestational periods in pregnancy to drugs of abuse were found in literature. Drug detection in maternal blood, oral fluid, and sweat accounts only for acute consumption that occurred in the hours previous to collection and gives poor information concerning fetal exposure [113].

Some characteristics of sweat such as the easy and noninvasive collection, the detection window of a few days before a patch application and the difficult to quantify the amount of sweat secreted allow this matrix to be considered for qualitative purposes [114]. Sweat specimens from pregnant opioid – dependent women (treated with methadone) were examined to detect methadone, cocaine and heroin metabolites [27, 74]. Correlation between patch concentrations and total amount of methadone administered and concentrations and duration of patch wear were both weak. Although there were large intra- and inter-subject variations in sweat drug concentrations, sweat testing was an effective alternative technique to qualitatively monitor illicit drug use and simultaneously document methadone medication-assisted treatment [27]. An opioid-depen dent buprenorphine-maintained pregnant woman [75] was submitted to weekly sweat patches application detecting buprenorphine, cocaine, opiates, methadone and tobacco biomarkers with good selectivity and sensitivity. 75.0% of sweat patches were positive for buprenorphine, 93.8% for cocaine, 37.5% for opiates, 6.3% for methadone and all for tobacco biomarkers. The pharmacokinetics of buprenorphine was studied [101] after high-dose sublingual tablet administration in three opioid-dependent pregnant women detecting buprenorphine and its metabolite in only four of 25 specimens in low concentrations (less than 2.4 ng/patch).

9. INTERPRETATION OF THE ANALYTICAL RESULTS

Duration of patch wear, variability in sweat production, and stability of drugs on the patch are some of the complex factors involved in interpretation of drug concentrations in this specimen. A major limitation of sweat patch testing [79] is that the production of liquid perspiration varies with ambient temperature and physical activity. Therefore the volume of perspiration collected by the patch worn during the week, is unknown. This precludes meaningful quantitative analysis of drugs detected on the patch and limits the interpretative value of a sweat patch drug test result. Moreover being the volume of sweat limited, preserving part of the specimen for an independent retest is difficult.

Despite of this consideration, SAMHSA issued mandatory guidelines [6] for the federal workplace drug testing programs for sweat testing. After chronic exposure lipophilic drugs may be stored in adipose tissue hence they do accumulate in fat falsely suggesting new episodes of drug intake. Interpretation of drug distribution and elimination in sebum and stratum corneum is complicated by possible contamination of specimens with drugs from sweat [2, 36], particularly because parent drug is often detected as the major analyte in the sweat patch.

Cone EJ. *et al.* [2] refer that during the course of the cocaine experiments, sweat patches were challenged in passive drug contamination studies (transdermal and cocaine vapor). The exterior environment during exposure was ruled out as an alternate explanation because the same subject showed negative results in other experiments. The authors concluded that environmental contamination of the sweat patch had most likely occurred during the removal and the storage process resulting in positive tests for cocaine. Obviously careful procedures must be employed in removal and handling of the sweat patches to prevent the production of false positive drug test results.

False positive interpretations may arise from prior presence of drugs on the exterior of the skin which are not removed by the cleaning process. Skin contamination experiments is distinct from drugs permeating the skin, entering the blood stream and being re-excreted by the sweat into the patch [13]. The presence of contaminants in the environment and hence the importance of the correct forensic interpretation was recently underlined by Daughton C.G. in 2011 [115]. Many drugs, especially illicit drugs, are readily excreted via sweat glands, including those on the fingers. This has the potential to result in contamination of samples during their collection or during various steps in analysis. Contamination of samples by analysts who are using prescribed or illicit drugs is an under-investigated potential source of erroneous data.

The presence of metabolites in sweat is thought to distinguish passive exposure from active use. The finding in skin wipes of unique metabolites of drugs that are not present in the environment would indicate use rather than exposure. The presence of heroin, 6-acetylmorphine, acetylcodeine allows unequivocal differentiation

between licit opiate use and heroin abuse [27]. The presence of cocaethylene or ecgonine methylester is thought to indicate the use of cocaine rather than exposure to it [16]. This unique metabolites are present in minor amounts requiring very sensitive techniques for detecting use versus exposure. Kidwell DA. *et al.* [16] stated that the benzoylecgonine/cocaine ratio varies widely. They also observed that some subjects showed a very high ratio, they speculated the presence of active enzymes on their skin or different excretory pathways for cocaine and its metabolites. However they performed experiments that showed cocaine stable in contact with the skin. This implies that the enzymes are not sufficiently active for substantial cocaine hydrolysis [16].

Kidwell DA. *et al.* [38] found positive results for an unknown period after cocaine cessation. In a legal setting the issue of when drug use occurs is crucial. For example, judges require drug abstinence as a standard condition of probationary release. Prior drug use may be irrelevant to meeting this condition. If the sweat patch is used as a stand-alone test for determining drug use status during the period that the sweat patch is worn, then it is essential that the patch does not falsely report previous drug use as "current drug use". For chronic users it is not clear whether a cocaine appearing in the patches came from current drug ingestion, previous drug ingestion, previous drug contamination, current drug contamination, or a combination of the above.

CONCLUSIONS

Forensic scientists have long detected the presence of drugs in biological materials using body fluids such as urine, blood, and/or tissues. In recent years, remarkable advances in sensitive analytical techniques have prompted the analysis of drugs in unconventional biological samples more easily collectable.

Patch technology allows the monitoring of illicit drug use for time windows wider than those provided by urine testing. Because the patches can be worn for up to one week, drugs tend to accumulate in the collection device, and no drug degradation appears to occur during this time interval. A series of clinical studies were designed to determine the identity, concentration, time course, dose dependency, and variability of drug and metabolite excretion in sweat following administration of single dose of illicit drugs to human subjects.

Although there are large intra- and inter-subject variations in sweat drug concentrations, sweat testing was found to be an effective alternative technique to qualitatively monitor illicit drug use and simultaneously document medication-assisted treatment.

Advantages of sweat analysis using sweat patches include: continuous drug testing can be undertaken over a longer period (up to 7-14 days) than urine or saliva, the process of specimens collection is less invasive than urine collection, the patches appear to be relatively tamper resistant and tamper evident, and the patch can be applied and removed quickly and little training is required for the sanitary. Moreover sweat patches are readily accepted by subjects limiting the number of required monitoring visits.

Disadvantages of sweat analysis using sweat patches limited routine use of this biological matrix. Concentrations of drugs in the patch are much lower than urine, making repeated testing (confirmation retesting) a potential problem. Possibility of environmental contamination of patch before application or after removal must be taken into account. Moreover there is the risk of accidental or deliberate removal of patch during monitoring period. The effects of vigorous or prolonged exercise on the transfer of drugs into sweat and / or the deposition of these drugs onto the patch are unknown and there is evidence that outward transdermal migration of some accumulated drugs may lead to an incorrect interpretation of new drug use. Finally the possibility of time-dependent drug loss from the patch by drug degradation on skin is also possible together with re-absorption into the skin and consequent volatile losses through the covering membrane of the patch.

Sweat patches provide a convenient alternative that avoids some of the problems with drug testing such as violations of privacy in observed urination, possibility of disease transmission, and transport of noxious fluids. This technology benefits from low invasiveness and pose fewer ethical problems for sample collection than does blood or urine testing. Nevertheless it would be premature to replace urine toxicology testing with sweat patch in both research and clinical settings. Continuing improvements in sweat collection and testing methods may result in the availability of a substantially improved sweat device in the near future.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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