Forensic Toxicol (2015) 33:12–36
DOI 10.1007/s11419-014-0258-1

REVIEW ARTICLE

Nail analysis for the detection of drugs of abuse and pharmaceuticals: a review

Delphine Cappelle · Michel Yegles · Hugo Neels · Alexander L. N. van Nuijs · Mireille De Doncker · Kristof Maudens · Adrian Covaci · Cleo L. Crunelle

Received: 31 October 2014 / Accepted: 14 November 2014 / Published online: 10 December 2014

Abstract Nails can stably accumulate substances for long periods of time, thus providing retrospective information regarding drugs of abuse and pharmaceutical use. Nails have several advantages over the conventional matrices, such as blood and urine, including a longer detection window (months to years), non-invasive sample collection, and easy storage and transport. These aspects make nails a very interesting matrix for forensic and clinical toxicology. Because of the low concentrations of drugs of abuse and pharmaceuticals present in nails and the complexity of the keratinized matrix, analytical methods need to be more sensitive, and sample preparation is crucial. This review summarizes the literature regarding the detection and quantification of drugs of abuse and pharmaceuticals in nails, as well as the employed pre-analytical and analytical techniques. Additionally, the applications of nail analysis are reviewed. Finally, an overview of the challenges of nail analysis is provided, and guidelines for future research are proposed.

Keywords Nail analysis · Drugs of abuse · Pharmaceuticals · Xenobiotics · Keratinized matrix · Pre-analytical and analytical techniques

D. Cappelle (✉) · H. Neels · A. L. N. van Nuijs · K. Maudens · A. Covaci · C. L. Crunelle
Toxicological Centre, University of Antwerp, CDE S.553, Universiteitsplein 1, 2610 Antwerp, Belgium
e-mail: delphine.cappelle@uantwerpen.be

M. Yegles
Laboratoire National de Santé, Service de Toxicologie, Dudelange, Luxembourg

H. Neels · M. De Doncker
Laboratory for TDM and Toxicology, ZNA Stuivenberg, Antwerp, Belgium

Introduction

Nails are keratinized matrices capable of storing substances for long periods of time [1, 2]. Since 1965, nails have been used to detect arsenic intoxication and exposure to metals such as cadmium, copper, lead, zinc, iron, and magnesium [3–7]. Over the years, nail analysis has expanded towards the detection of drugs of abuse, pharmaceuticals, and their metabolites. For example, nails have been employed for the therapeutic drug monitoring of antimycotics and for the detection of amphetamine-like substances, cocaine, and opiates [1, 8].

Incorporation of substances into nails mainly occurs through diffusion from the rich blood supply, which deposits substances to both the germinal matrix and the nail bed on the underside of the nail plate, thus allowing incorporation in both a vertical and horizontal way during nail formation [9] (see Fig. 1). Other mechanisms of incorporation that have been proposed are incorporation through diffusion from biological fluids such as sweat, sebum, and saliva [10–12], and incorporation

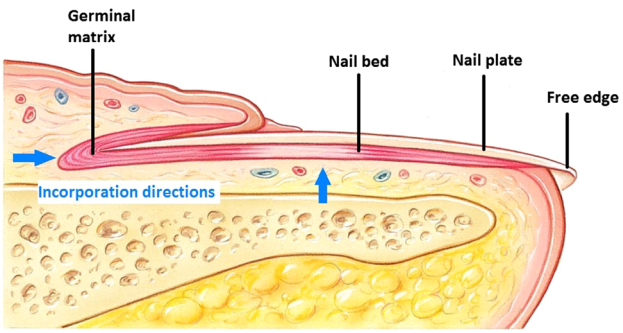

Fig. 1 Structure of the nail: a sagittal section of the fingertip (adapted from [14])

through diffusion from the external environment [13]. However, this latter pathway of incorporation is minimal [13].

Nails grow at a continuous rate. The average growth rate of fingernails is 3.0 mm per month, while toenails grow at an average rate of 1.1 mm per month [9, 14, 15]. The regeneration time (i.e., the time to grow from the germinal matrix to the nail's free edge) is 3–5 and 8–16 months for finger- and toenails, respectively [1, 15, 16].

Compared to blood and urine, nails have a longer window of detection (months to years) from which information on xenobiotic exposure or ingestion can be retrieved [1, 2, 17]. Nail collection is non-invasive, easy to perform, does not require medically trained personnel, and can be achieved under close supervision to prevent adulteration. Additionally, nails can be transported and stored at room temperature. These advantages are similar to hair, another keratinized matrix that has been used for several years to assess retrospectively drug and pharmaceutical use [18]. Interestingly, nails provide some additional advantages over hair. Firstly, when hair is not (sufficiently) available (e.g., with alopecia, during chemotherapy, or during the first weeks or months after birth), nail analysis can be an important tool to gain retrospective information on xenobiotic use. Secondly, in contrast to hair, nails do not contain melanin. Since drug incorporation may be influenced by melanin concentrations [19, 20], hair pigmentation becomes an important source of bias when interpreting detected drug concentrations. Thirdly, nails grow slower than hair, which provides the opportunity to detect smaller exposure levels and/or to investigate longer periods of time. Fourthly, while hair is characterized by a cyclic growth rate with different stages, nails grow at a constant rate, which facilitates the interpretation of results. Finally, compared to hair sampling, nail collection is esthetically more acceptable, easier, and less intrusive. Taken together, these advantages underline the potential of nails as an interesting and useful matrix for the retrospective detection of drug and pharmaceutical use.

Because of the complexity of the keratinized matrix and the low concentrations that have to be measured (pg/mg to ng/mg range), an extensive sample preparation procedure as well as a specific and sensitive analytical technique are required (see Fig. 2). The sample preparation procedure comprises the following steps: decontamination, homogenization, extraction, and clean-up. Decontamination involves the use of small volumes of washing solvents (e.g., water, acetone, and methanol) for a few minutes at room temperature. Samples are then dried, weighed, cut into small pieces, and pulverized. Subsequently, substances need to be extracted from the nail matrix by either direct extraction or digestion. Further clean-up, usually through liquid–liquid extraction (LLE) or solid-phase extraction (SPE), is necessary to eliminate interferences and concentrate the compounds of interest. Most of the analytical methods for nail analysis are based on gas chromatography (GC) or liquid chromatography (LC) coupled to mass spectrometry (MS) or tandem MS (MS–MS) to achieve sufficient specificity and sensitivity.

Fig. 2 Pre-analytical and analytical steps of nail analysis

The aims of this review are fourfold: (i) to review the literature regarding drugs of abuse and pharmaceuticals detected in nails, including the pre-analytical and analytical techniques used, (ii) to give an overview of the applications in which nails are used, (iii) to provide a brief comparison between nail and hair analysis, and (iv) to address the current issues of nail analysis and challenges for future research.

Literature search

The literature of nail analysis was reviewed up to October 2014. Publications related to the detection and quantification of drugs in nails were searched in Pubmed and Web of Science using combinations of the search terms "nail", "drug", "abuse", "forensic toxicology", "pharmaceutical","antimycotic", "doping", "amphetamine", "cocaine", "morphine", "cannabi", "ketamine", "caffeine", "nicotine", "steroid", and "ethylglucuronide". A comprehensive database of retrieved articles was built through direct search and cross-references. Articles were limited to the English language. Duplicates and articles judged not pertinent to the topic (e.g., articles on dermatology, nail disorders, and the detection of elements) were excluded.

Amphetamines

Table 1 provides an overview of the pre-analytical and analytical techniques used for the detection of pharmaceuticals and organic toxic substances in nails. The main findings of the studies detecting them in nails are summarized in Table 2.

Pre-analytical and analytical techniques

Gas chromatography coupled to mass spectrometry (GC–MS) operated in electron ionization (EI) mode has been the preferred technique for the detection of methamphetamine (mAMP), amphetamine (AMP), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyamphetamine (MDA) in nails [16, 21–25]. Decontamination consisting of water and methanol washes was found to be efficient as no substances were detected in a methanol wash performed after the normal decontamination procedure [23]. Washing has usually been followed by alkaline extraction and further clean-up with liquid–liquid extraction (LLE) [8, 16, 22–24]. In recent years, a rapid and easy sample preparation procedure was proposed in which decontamination was immediately followed by mechanical pulverization of the nail, methanol extraction, and purification by high speed centrifugation [25]. Thereby, alkaline extraction, which can result in the loss of amphetamines by evaporation, was avoided. Moreover, compared to the extracts achieved by alkaline hydrolysis and subsequent LLE, the procedure resulted in cleaner extracts and enhanced detection sensitivity of amphetamines, with low limits of quantification (LOQs) (e.g., 0.05 ng/mg for MDMA) [25].

Amphetamine detection and quantification in nails

In the earliest study by Suzuki et al. [8], mAMP and its metabolite AMP were detected in finger- and toenail clippings from nine mAMP users, with values ranging from 0.06 to 17.7 ng/mg for mAMP and from 0.03 to 1.60 ng/mg for AMP. In three subjects from which both finger- and toenails were available, higher mAMP and AMP concentrations were found in toenails than in fingernails [8]. In addition, the authors scraped off the underside layer of the clippings (removing approximately 20 % of the nail weight) to evaluate external contamination through sweat, but did not observe differences between mAMP and AMP concentrations in scraped and non-scraped clippings [8]. Another study by Suzuki et al. [21] reported that in nail samples from 20 mAMP users, mAMP was detected more frequently and at higher concentrations than its metabolite AMP; mAMP was detected in 13 out of 20 samples from 0.4 to 642 ng/mg, whereas AMP was detected in only three out of 20 samples from 0.3 to 23.2 ng/mg [21]. They emphasized the potential of nails for retrospective detection by demonstrating that substances could be detected for longer periods in nails (45 days for mAMP) as compared to saliva (2 days for mAMP) and sweat. The longer detection window of nails was further confirmed by a study in which high MDMA concentrations in nails (60.2 ng/mg) revealed chronic MDMA consumption, which was not evident from results obtained by blood and urine analysis (no detection of MDMA) [22]. In nine samples of multi-drug users, mAMP and AMP were detected in 30 % of the cases with values ranging from 1.00 to 1.41 ng/mg and from 0.12 to 2.64 ng/mg, respectively [23]. Fingernails of seven drug abusers were analyzed for mAMP (six positive cases; 0.23–2.09 ng/mg), AMP (four positive cases; <0.063 ng/mg), MDA (one positive case; <0.143 ng/mg), and MDMA (one positive case; 0.46 ng/mg) [24]. In toenails of four drug abusers, mAMP and AMP were both detected in one case (concentrations not provided) [25]. A larger study in 97 female AMP and/or opiate drug users, who were currently under treatment, reported the duration of detectability and deposition characteristics of mAMP and AMP in fingernails [16]; 62 subjects were found positive for mAMP and/or AMP, with concentrations ranging from 0.46 to 61.5 ng/mg and from <0.2 to 5.42 ng/mg, respectively. Distribution patterns from samples of eight subjects collected every 4 weeks over a period of 12 weeks showed that drugs are not only deposited at the germinal matrix, but also along the length of the nail bed. In four out of eight analyzed samples, mAMP was below the limit of detection (LOD) 8 weeks after the first sample collection [16].

Discussion

Amphetamines, among the first drugs of abuse investigated in nails, are detected presumably with higher concentrations in toenails than in fingernails. This was hypothesized to be due to a slower growth rate of toenails as compared to fingernails. However, only a single and relatively small study [8] compared the levels of amphetamines in fingernails with toenails; thus more research is needed. Studies on amphetamines in nails have provided evidence that nails have a relatively long window of detection and could potentially be used to retrieve information about long-term consumption, even if relatively low concentrations are detected in nails. Whether a single use of AMP, mAMP, MDA, and/or MDMA can also be detected in nails is still unknown.

Cocaine

Pre-analytical and analytical techniques

GC–EI–MS has been used throughout the studies for the detection of cocaine and its metabolites in nails [10, 13,

Table 1 Overview of the pre-analytical and analytical techniques for the detection of drugs of abuse and pharmaceuticals in nails

Reference	Substance(s)	Washing procedure	Sample weight (mg)	Homogenization	Extraction	Clean-up	Derivatization	Technique	LOD	LOQ
Amphetamines and ketamine										
[8]	(m)AMP	MeOH/water (1:1, v/v), ultrasonication	Not mentioned	None	2.5 M NaOH; 80 °C, 30 min	LLE	TFAA	GC–CI–MS	10 pg on column	Not provided
[21]	(m)AMP	5 × water; 5 × MeOH, alternated	10–30	Crushed	0.6 M HCl; not mentioned	LLE	TFAA	GC–EI–MS	20 pg on column	Not provided
[22]	AMP, MD(M)A	DCM	14	Scraped	1 M NaOH; 95 °C, 10 min	LLE	PFPA + PFP	GC–EI–MS	Not provided	Not provided
[16]	(m)AMP	MeOH	Not mentioned	None	2 M NaOH; 80 °C, 1 h	LLE	HFBA	GC–EI–MS	0.2 ng/mg	0.2 ng/mg
[23]	(m)AMP, MD(M)A, THC, THC-COOH	1 × water; 3 × MeOH	30	None	1 M NaOH; 95 °C, 30 min	LLE	MSTFA	GC–EI–MS	0.016–0.056 ng/mg	0.1–0.2 ng/mg
[24]	(m)AMP, MD(M)A, ketamine, norketamine	1 × water; 2 × MeOH	20	None	1 M NaOH; 95 °C, 30 min	LLE	HFBA	GC–EI–MS	0.015–0.094 ng/mg	0.05–0.314 ng/mg
[25]	(m)AMP, MD(M)A, norketamine	1 × water; 3 × MeOH	20	Pulverized	MeOH; 50 °C, 1 h, ultrasonication	None	HFBA	GC–EI–MS	0.012–0.024 ng/mg	0.05–0.08 ng/mg
Cocaine and opioids										
[27]	6-MAM, morphine, cocaine, benzoylecgonine, codeine	2 × MeOH	10.1	Pulverized	MeOH; 4 h, ultrasonication	None	PFPA + HFIP	GC–EI–MS	0.1–0.2 ng/mg	Not provided
[28]	Cocaine, benzoylecgonine, norcocaine, cocaethylene, morphine, 6-MAM, codeine, hydrocodone	3 × MeOH	100	Cut	0.1 M Phosphate buffer (pH 5); 1 h, sonication + room temp, 72 h, soaked	SPE	MSTFA	GC–EI–MS	Not provided	0.3 ng on column
[29]	Cocaine, benzoylecgonine, norcocaine, norbenzoylecgonine, cocaethylene, EEE, EME, AEME	MeOH	7–25	Cut	MeOH; 40 °C, 16 h, reflux	SPE	BSTFA + TMCS	GC–EI–MS	0.10–0.25 ng/mg	0.25–0.50 ng/mg
[36]	Morphine	1 × SDS; 3 × water; 3-4 × MeOH, ultrasonication	3–96	None	1 M NaOH; 60 °C, 1-2 h	LLE	None	LC–ECD	0.05 ng/mg	Not provided
[37]	Methadone	1 × SDS; 3 × water; 3 × MeOH, ultrasonication	0.18–16.33	None	1 M NaOH; 90 °C, 30-40 min	SPE	None	GC–EI–MS	0.005 ng/mg	Not provided
[10]	Cocaine, codeine	1 × iPrOH, 3 × phosphate buffer (pH 6), agitation	15	Scraped	Enzymatic digestion (protease XI, dithiothreitol, Tris buffer); 40 °C, overnight	SPE	BSTFA + TMCS	GC–EI–MS	Not provided	0.13–1.0 ng/mg
[13]	Cocaine, benzoylecgonine, norcocaine, EME, cocaethylene, morphine, 6-MAM, codeine, hydromorphone, oxycodone, hydrocodone	3 × MeOH	20–30 (cocaine); 100–200 (opioids)	None	0.1 M Phosphate buffer (pH 5); 1 h, sonication + room temp, 72 h, soaked	SPE (cocaine); LLE (opioids)	MSTFA	GC–EI–MS	Not provided	0.1 ng on column

Table 1 continued

Reference	Substance(s)	Washing procedure	Sample weight (mg)	Homogenization	Extraction	Clean-up	Derivatization	Technique	LOD	LOQ
[26]	Cocaine, morphine, 6-MAM	2 × DCM	50	Cut	37 % HCl (v/v in water); 100 °C, 30 min + room temp, 12 h	LLE + SPE	Propionic anhydride	GC–EI–MS	Not provided	0.1 ng/mg
[30]	Cocaine, AEME, EME, cocaethylene, benzoylecgonine	1 × hot water; 5 × DCM	50	Pulverized	1 M HCl; 56 °C, overnight	LLE	BSTFA + TMCS	GC–EI–MS	0.05–0.50 ng/mg	Not provided
[31]	Cocaine, benzoylecgonine, norcocaine	3 × MeOH	5	Cut	MeOH; 40 °C, 16 h	SPE	PFPA + PFP	GC–EI–MS	3–3.5 ng/mg	Not provided
[32]	Cocaine, benzoylecgonine, morphine, methadone, caffeine, nicotine, cotinine	MeOH	10	Cut	1 M HCl; overnight	SPE	BSTFA + TMCS	GC–EI–MS	0.025 ng/mg	0.025–0.05 ng/mg
[38]	6-MAM, morphine, codeine, acetylcodeine, heroine	2 × water; 2 × acetone	20	Pulverized while cooled	Borate buffer (pH 9.2); room temp, 30 min, ultrasonication	LLE	None	LC–ESI–MS–MS	0.01–0.03 ng/mg	0.05 ng/mg
Cannabinoids										
[40]	THC, THC-COOH	1 × SDS; 3 × water; 3 × MeOH, sonication	2.5–12.3	None	1 M NaOH; 95 °C, 30 min	LLE	BSTFA + TMCS	GC–EI–MS	0.1 ng/mg	Not provided
[39]	THC-COOH	DCM	10–50	None	1 M NaOH; 80 °C, 1 h	SPE	PFPA + HFIP	GC–NICI–MS–MS	0.01 pg/mg	0.02 pg/mg
Alcohol										
[42, 43]	EtG	DCM, ultrasonication; MeOH	30	None	Water; 2 h, ultrasonication	None	None	LC–ESI–MS–MS	3 pg/mg	10 pg/mg
[41, 45]	EtG	Hexane; DCM; MeOH	5–50	Pulverized	Water; 40 °C, 2 h, ultrasonication + room temp, overnight	SPE	None	LC–ESI–MS–MS	2 pg/mg	8 pg/mg
[44]	EtG	None	50	Pulverized	ACN/water (1:1); 25 °C, 2 h, ultrasonication	LLE	None	LC–ESI–MS–MS	0.48 pg/mg	1.61 pg/mg
Phencyclidine										
[47]	Phencyclidine	3 × MeOH	100–200	Cut	1 M NaOH; 1 h, ultrasonication + room temp, overnight	LLE	None	GC–EI–MS	Not provided	Not provided
Caffeine and nicotine										
[49, 52, 53, 55]	Nicotine	DCM	10–30	Cut	1 M NaOH; 50 °C, overnight	LLE	None	LC–ECD	0.01 ng/mg	Not provided
[50, 51]	Nicotine, cotinine	DCM (room temp, 2 h)	20–30	None	1 M NaOH; 50 °C, overnight	LLE	None	GC–EI–MS	0.01–0.012 ng/mg	Not provided
[50, 51]	NNAL	DCM (room temp, 2 h)	50–80	None	1 M NaOH; 50 °C, overnight	2 × SPE	None	LC–ESI–MS–MS	0.02 pg/mg	Not provided
[57]	NNN	DCM (room temp, 2 h)	40–100	None	1 M NaOH; 50 °C, overnight	3 × SPE	None	LC–ESI–MS–MS	0.02 pg/mg in a 50 mg sample	Not provided
[54]	Nicotine, cotinine, myosmine	DCM (room temp, 2 h)	20–30	Cut	1 M NaOH; 50 °C, overnight	3 × LLE	None	GC–EI–MS	0.01–0.035 ng/mg	0.03–0.105 ng/mg
[56]	Nicotine	None	≥20	None	None	LLE	None	GC–EI–MS–MS	Not provided	0.005 ng/mg

Table 1 continued

Reference	Substance(s)	Washing procedure	Sample weight (mg)	Homogenization	Extraction	Clean-up	Derivatization	Technique	LOD	LOQ
Sedative and antipsychotic drugs										
[59]	Alprazolam, clobazam, clonazepam, diazepam, lorazepam, midazolam, oxazepam, temazepam, triazolam, zopiclone	Water; 3 × EtOH	Not mentioned	Cut	TFA/MeOH (1:50); room temp, 16-18 h	LLE	None	LC–ESI–MS–MS	0.06–6 pg/mg	Not provided
[11]	Zolpidem	1 × water; 2 × MeOH	25	Cut	0.3 M NaOH; 80 °C, 40 min	LLE	None	LC–ESI–MS–MS	0.03 pg/mg	0.05 pg/mg
[12]	Zolpidem	None	5–10	Pulverized	MeOH; 6 h, ultrasonication	None	None	LC–ESI–MS–MS	Not provided	0.1 pg/mg
[60]	Clozapine	2 × water; 1 × acetone	5	Pulverized while cooled	70 % ACN–30 % ammonium acetate buffer; room temp, 1 h, ultrasonication	None	None	LC–ESI–MS–MS	0.05 ng/mg	0.5 ng/mg
[61]	Clozapine	2 × water; 2 × ethyl acetate	5	Pulverized while cooled	70 % ACN–30 % ammonium acetate buffer; room temp, 1 h, ultrasonication	None	None	UHPLC–ESI–MS–MS	Not provided	10 pg/mg
Steroids										
[62]	Testosterone, pregnenolone	3 × MeOH	100	Cut	1 M NaOH; 80 °C, 1 h	LLE	Flophemesyl-TMS	GC–EI–MS	0.1 ng/g	0.2–0.3 ng/g
[63]	Testosterone, testosterone propionate, stanazol	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Not mentioned	LC-ESI-MS	Not determined	Not determined
General unknown screening										
[64]	General unknown screening	2 × water; 2 × acetone	20	Pulverized	2 × MeOH, ACN, water with ammonium formate (25:25:50, v/v/v); 40 °C, 18 h	None	None	LC–ESI–QTOF–MS		No quantification

ACN acetonitrile, *AEME* anhydroecgonine methyl ester, *AMP* amphetamine, *BSTFA N,O*-bis(trimethylsilyl)trifluoroacetamide, *CI* chemical ionization, *DCM* dichloromethane, *ECD* electrochemical detection, *EEE* ecgonine ethyl ester, *EME* ecgonine methyl ester, *ESI* electron spray ionisation, *EtG* ethyl glucuronide, *EtOH* ethanol, *flophemesyl-TMS* pentafluorophenyl dimethylsilyl-trimethylsilyl, *GC* gas chromatography, *HCl* hydrochloric acid, *HFBA* heptafluorobutyric anhydride, *HFIP* hexafluoroisopropanol, *iPrOH* isopropanol, *LC* liquid chromatography, *LLE* liquid–liquid extraction, *LOD* limit of detection, *LOQ* limit of quantification, *6-MAM* 6-monoacetylmorphine, *mAMP* methamphetamine, *MDA* 3,4-methylenedioxyamphetamine, *MDMA* 3,4-methylenedioxymethamphetamine, *MeOH* methanol, *MS(-MS)* mass spectrometry (tandem), *MSFTA N*-methyl-*N*-trimethylsilyltrifluoroacetamide, *NaOH* sodium hydroxide, *NICI* negative ion chemical ionisation, *NNAL* 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, NNN *N*-nitrosonornicotine, *PFP* pentafluoropropionyl, *PFPA* pentafluoropropionic anhydride, *QTOF* quadrupole time-of-flight, *SDS* sodium dodecyl sulphate, *SPE* solid-phase extraction, *TFA* trifluoroacetic acid, *TFAA* trifluoroacetic anhydride, *THC* Δ^9-tetrahydrocannabinol, *THC-COOH* 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol, *TMCS* trimethylchlorosilane, *UHPLC* ultra high-performance liquid chromatography

Table 2 Overview of the studies detecting drugs of abuse and pharmaceuticals in nails and their main findings

Reference	Population	Sample size (mg)	Sample type	Substances detected (positive samples/total samples)	Concentration range in (positive) samples	LOD	LOQ
Amphetamines and ketamine							
[8]	Methamphetamine users	Not mentioned	Separate finger- and toenail clippings	mAMP F (7/8)	0.32 to 17.70 ng/mg	10 pg on column	Not provided
				mAMP T (4/4)	0.06 to 9.93 ng/mg		
				AMP F (5/8)	0.03 to 0.40 ng/mg		
				AMP T (3/4)	0.03 to 1.60 ng/mg		
[21]	Methamphetamine users	10–30	Not mentioned	mAMP (13/20)	0.4 to 642.0 ng/mg	20 pg on column	Not provided
				AMP (3/20)	0.3 to 23.2 ng/mg		
[22]	Drug abuser	14	Fingernail scrapings	AMP (1/1)	12.0 ng/mg	Not provided	Not provided
				MDA (1/1)	9.7 ng/mg		
				MDMA (1/1)	60.2 ng/mg		
[16]	Amphetamine and/or opiate users	Not mentioned	Fingernail clippings	mAMP (62/97)	0.46 to 61.50 ng/mg	0.2 ng/mg	0.2 ng/mg
				AMP (50/97)	<LOD to 5.42 ng/mg		
[23]	Drug abusers	30	Fingernail clippings	mAMP (3/9)	1.00 to 1.41 ng/mg	0.025 ng/mg	0.2 ng/mg
				AMP (2/9)	0.12 and 2.64 ng/mg	0.016 ng/mg	0.1 ng/mg
				MDA (0/9)	ND	0.043 ng/mg	0.2 ng/mg
				MDMA (0/9)	ND	0.044 ng/mg	0.2 ng/mg
				THC (0/9)	ND	0.056 ng/mg	0.2 ng/mg
				THC-COOH (1/9)	0.20 ng/mg	0.035 ng/mg	0.2 ng/mg
[24]	Drug abusers	20	Fingernail clippings	mAMP (6/7)	0.23 to 2.09 ng/mg	0.044 ng/mg	0.147 ng/mg
				AMP (4/7)	<LOQ	0.019 ng/mg	0.063 ng/mg
				MDA (1/7)	<LOQ	0.043 ng/mg	0.143 ng/mg
				MDMA (1/7)	0.46 ng/mg	0.016 ng/mg	0.053 ng/mg
				Ketamine (1/7)	<LOQ	0.094 ng/mg	0.314 ng/mg
				Norketamine (1/7)	<LOQ	0.015 ng/mg	0.050 ng/mg
[25]	Drug abusers	20	Toenail clippings	mAMP (1/4)	Not provided	0.024 ng/mg	0.08 ng/mg
				AMP (1/4)	Not provided	0.015 ng/mg	0.05 ng/mg
				MDA (0/4)	ND	0.014 ng/mg	0.05 ng/mg
				MDMA (0/4)	ND	0.012 ng/mg	0.05 ng/mg
				Norketamine (0/4)	ND	0.014 ng/mg	0.05 ng/mg
Cocaine and opioids							
[27]	Postmortem sample from a 3-month old child	10.1	Combined finger- and toenail clippings	Cocaine (1/1)	~0.3 ng/mg	0.1 ng/mg	Not provided
				Benzoylecgonine, 6-MAM, morphine (0/1)	ND	0.1 ng/mg	
				Codeine (0/1)	ND	0.2 ng/mg	

Table 2 continued

Reference	Population	Sample size (mg)	Sample type	Substances detected (positive samples/total samples)	Concentration range in (positive) samples	LOD	LOQ
[28]	Postmortem samples	100	Toenail clippings	Cocaine (20/46)	0.20 to 140.17 ng/mg	Not provided	0.3 ng on column
				Benzoylecgonine (21/46)	0.30 to 315.44 ng/mg		
				Norcocaine (2/46)	0.66 and 6.78 ng/mg		
				Cocaethylene (2/46)	0.73 and 2.60 ng/mg		
				Morphine (3/34)	0.16 to 0.72 ng/mg		
				6-MAM (3/34)	0.41 to 1.70 ng/mg		
				Codeine (2/34)	1.02 and 3.07 ng/mg		
				Hydrocodone (1/34)	0.62 ng/mg		
[29]	Postmortem samples from cocaine users	7–25	Separate finger- and toenail clippings	Cocaine F (14/17)	<LOD to >10 ng/mg	0.10 ng/mg	0.25 ng/mg
				Cocaine T (8/15)	<LOD to >10 ng/mg		
				Benzoylecgonine F (7/17)	<LOD to >10 ng/mg	0.10 ng/mg	0.25 ng/mg
				Benzoylecgonine T (7/15)	<LOD to >10 ng/mg		
				Norcocaine F (5/17)	<LOD to 6.36 ng/mg	0.10 ng/mg	0.25 ng/mg
				Norcocaine T (4/15)	<LOD to 0.87 ng/mg		
				Norbenzoylecgonine F (3/17)	0.61 to 2.59 ng/mg	0.25 ng/mg	0.50 ng/mg
				Norbenzoylecgonine T (1/15)	<LOQ		
				Cocaethylene F (1/17)	<LOQ	0.10 ng/mg	0.25 ng/mg
				Cocaethylene T (1/15)	1.03 ng/mg		
				EEE F (0/17)	ND	0.25 ng/mg	0.50 ng/mg
				EEE T (1/15)	<LOQ		
				EME F (3/17)	0.87 to >10 ng/mg	0.10 ng/mg	0.25 ng/mg
				EME T (4/15)	<LOD to 1.18 ng/mg		
				AEME F (4/17)	<LOD to >10 ng/mg	0.10 ng/mg	0.25 ng/mg
				AEME T (4/15)	<LOD to 1.63 ng/mg		
[36]	Heroin users	3–96	Fingernail clippings	Morphine (22/26)	0.14 to 6.90 ng/mg	0.05 ng/mg	Not provided
[37]	Patients following a methadone treatment	0.18–16.33	Fingernail clippings	Methadone (27/29)	0.51 to 362.50 ng/mg	0.005 ng/mg	Not provided
[10]	Previous drug users ingesting low/high dose of cocaine/codeine	15	Fingernail scrapings	Cocaine 75 mg/70 kg (8/8)	0.25 to 1.60 ng/mg	Not provided	0.13–1.0 ng/mg
				Cocaine 150 mg/70 kg (7/8)	0.57 to 2.70 ng/mg		
				Codeine 60 mg/70 kg (4/8)	0.12 to 0.31 ng/mg		
				Codeine 120 mg/70 kg (6/8)	0.12 to 0.20 ng/mg		

Table 2 continued

Reference	Population	Sample size (mg)	Sample type	Substances detected (positive samples/total samples)	Concentration range in (positive) samples	LOD	LOQ
[13]	Postmortem samples from drug abusers and/or criminal offenders	20–30 (cocaine); 100–200 (opioids)	Separate finger- and toenail clippings (left and right)	Cocaine F (15/15)	6.0 to 414.1 ng/mg	Not provided	0.1 ng on column
				Cocaine T (14/15)	1.2 to 19.9 ng/mg		
				Benzoylecgonine F (15/15)	1.9 to 170.3 ng/mg		
				Benzoylecgonine T (15/15)	1.4 to 27.0 ng/mg		
				EME F (14/15)	0.4 to 27.0 ng/mg		
				EME T (11/15)	0.2 to 3.6 ng/mg		
				Norcocaine F (12/15)	0.2 to 32.7 ng/mg		
				Norcocaine T (7/15)	0.1 to 0.9 ng/mg		
				Cocaethylene F (2/15)	<LOQ to 2.9 ng/mg		
				Cocaethylene T (2/15)	0.1 to 1.9 ng/mg		
				Morphine F (10/16)	0.08 to 407.9 ng/mg		
				Morphine T (11/16)	0.05 to 6.9 ng/mg		
				6-MAM F (10/16)	0.19 to 504.0 ng/mg		
				6-MAM T (9/16)	0.04 to 13.1 ng/mg		
				Codeine F (7/16)	0.23 to 6.1 ng/mg		
				Codeine T (5/16)	0.06 to 8.8 ng/mg		
				Hydromorphone F (1/16)	0.21 and 0.28 ng/mg		
				Hydromorphone T (2/16)	0.12 to 0.45 ng/mg		
				Oxycodone F (1/16)	5.05 and 6.58		
				Oxycodone T (1/16)	6.36 and 6.88 ng/mg		
[26]	Postmortem samples from cocaine or heroin users	50	Entire (big toe) nail plates	Cocaine (7/18)	LOQ to 4.60 ng/mg	Not provided	0.1 ng/mg
				Morphine (13/18)	0.41 to 3.05 ng/mg		
				6-MAM (5/18)	0.25 to 0.83 ng/mg		
[30]	Homicide suspects	50	Fingernail clippings	Cocaine (2/2)	28.7 and 34.5 ng/mg	0.05 ng/mg	Not provided
				Benzoylecgonine (2/2)	7.3 and 17.9 ng/mg	0.15 ng/mg	
				EME (2/2)	2.5 and 6.3 ng/mg	0.50 ng/mg	
				Cocaethylene (0/2)	<LOD ng/mg	0.05 ng/mg	
				AEME (2/2)	0.24 and 0.39 ng/mg	0.10 ng/mg	
[31]	Cocaine users	5	Separate finger- and toenail clippings	Cocaine (1/8), benzoylecgonine (5/8)	Not provided	3.0 ng/mg	Not provided
				Norcocaine (2/8)		3.5 ng/mg	

Table 2 continued

Reference	Population	Sample size (mg)	Sample type	Substances detected (positive samples/total samples)	Concentration range in (positive) samples	LOD	LOQ
[32]	Newborns abandoned at birth (25), newborns from maternity hospital (33)	10	Combined finger- and toenail clippings	Caffeine (12/25) (8/33), nicotine (13/25) (10/33), cotinine (13/25) (9/33)	Not quantified	0.025 ng/mg	Not quantified
				Cocaine (6/25) (0/33)	0.14 to 0.25 ng/mg	0.025 ng/mg	0.025 ng/mg
				Benzoylecgonine (6/25) (0/33)	0.12 to 0.20 ng/mg	0.025 ng/mg	0.025 ng/mg
				Morphine (4/25) (0/33)	0.09 to 0.15 ng/mg	0.025 ng/mg	0.025 ng/mg
				Methadone (5/25) (2/33)	0.12 to 0.26 ng/mg	0.025 ng/mg	0.050 ng/mg
[38]	Subjects whose urine tested positive for morphine	20	Fingernail clippings	Morphine (12/18)	0.58 to 3.16 ng/mg	0.02 ng/mg	0.05 ng/mg
				6-MAM (12/18)	0.10 to 1.37 ng/mg	0.01 ng/mg	0.05 ng/mg
				Codeine (12/18)	<LOQ to 0.27 ng/mg	0.03 ng/mg	0.05 ng/mg
				Acetylcodeine (1/18)	<LOQ	0.01 ng/mg	0.05 ng/mg
				Heroin (3/18)	<LOQ	0.01 ng/mg	0.05 ng/mg
Cannabinoids							
[40]	Cannabis users	2.5–12.3	Fingernail clippings	THC (11/14)	0.13 to 6.97 ng/mg	0.1 ng/mg	Not provided
				THC-COOH (2/3)	9.82 and 29.67 ng/mg	0.1 ng/mg	
[39]	From a previous study Jones et al. 2012	10–50	Fingernail clippings	THC-COOH (32/60)	<LOD to 0.052 pg/mg	0.01 pg/mg	0.02 pg/mg
Alcohol							
[42]	Non-drinkers, social drinkers, heavy drinker	30	Fingernail clippings	EtG in non-drinkers (0/10)	<LOQ	3 pg/mg	10 pg/mg
				EtG in social drinkers (4/4)	12.3 to 84.3 pg/mg		
				EtG in heavy drinker (1/1)	92.6 pg/mg		
[43]	Mothers of newborns in neonatal wards	Not mentioned	Fingernail clippings	EtG (0/151)	<LOQ	3 pg/mg	10 pg/mg
[41]	Undergraduate college students	10–50	Fingernail clippings	EtG (203/529)	<LOD to 397 pg/mg	2 pg/mg	8 pg/mg
[45]	From a previous study Jones et al. 2012	≥5	Fingernail clippings	EtG (?/447)	<LOD to 397 pg/mg	2 pg/mg	8 pg/mg
[44]	Individuals presented for forensic examination	50	Fingernail clippings	EtG in non-drinkers (0/4)	ND	0.48 pg/mg	1.61 pg/mg
				EtG in drinkers (12/12)	5.97 to 90.52 pg/mg		
Phencyclidine							
[47]	Postmortem samples from homicide victims	100–200	Separate finger- and toenail clippings	Phencyclidine F (3/3)	4.13 to 147.9 ng/mg	Not provided	Not provided
				Phencyclidine T (4/4)	0.33 to 9.74 ng/mg		

Table 2 continued

Reference	Population	Sample size (mg)	Sample type	Substances detected (positive samples/total samples)	Concentration range in (positive) samples	LOD	LOQ
Caffeine and nicotine							
[49]	Smoking and non-smoking female nurses	10–30	Toenail clippings	Nicotine in non-smokers (25/25)	Mean: 0.08 ng/mg	0.01 ng/mg	Not provided
				Nicotine in ETS (29/29)	Mean: 0.28 ng/mg		
				Nicotine in smokers (25/25)	Mean: 1.71 ng/mg		
				Nicotine in smokers + ETS (26/26)	Mean: 2.18 ng/mg		
[52]	Smoking and non-smoking female nurses	10–30	Toenail clippings	Nicotine in non-smokers (157/157)	Median: 0.10 ng/mg	0.01 ng/mg	Not provided
				Nicotine in non-smokers + ETS (768/768)	Median: 0.14 ng/mg		
				Nicotine in smokers (771/771)	Median: 1.77 ng/mg		
[53]	Follow-up of female nurses developing CHD	10–30	Toenail clippings	Nicotine in CHD patients (905/905)	Mean ± SD: 1.38 ± 2.2 ng/mg	0.01 ng/mg	Not provided
				Nicotine in controls (1810/1810)	Mean ± SD: 0.70 ± 1.3 ng/mg		
[55]	Follow-up of males developing lung cancer	10–30	Toenail clippings	Nicotine in lung cancer patients (210/210)	Mean ± SE: 0.95 ± 0.09 ng/mg	0.01 ng/mg	Not provided
				Nicotine in controls (630/630)	Mean ± SE: 0.25 ± 0.02 ng/mg		
[50]	Smokers and non-smokers	20–30 (nicotine, cotinine); 50–80 (NNAL)	Toenail clippings	Nicotine in smokers (35/35)	Mean ± SD: 5.9 ± 5.6 ng/mg	0.01 ng/mg	Not provided
				Nicotine in non-smokers (6/6)	Mean: 0.09 ng/mg		
				Cotinine in smokers (35/35)	Mean ± SD: 1.6 ± 1.3 ng/mg	0.012 ng/mg	
				Cotinine in non-smokers (6/6)	Mean: 0.01 ng/mg		
				NNAL in smokers (35/35)	Mean ± SD: 0.41 ± 0.67 pg/mg	0.02 pg/mg	
				NNAL in non-smokers (0/6)	ND		
[51]	Smokers	20–30 (nicotine, cotinine); 50–80 (NNAL)	Toenail clippings	Nicotine (105/105)	0.01 to 33.18 ng/mg	0.01 ng/mg	Not provided
				Cotinine (105/105)	< LOD to 3.87 ng/mg	0.012 ng/mg	
				NNAL (51/51)	< LOD to 1.10 pg/mg	0.02 pg/mg	

Table 2 continued

Reference	Population	Sample size (mg)	Sample type	Substances detected (positive samples/total samples)	Concentration range in (positive) samples	LOD	LOQ
[57]	Smokers and non-smokers	40–100	Toenail clippings	NNN in smokers (16/17)	Mean ± SD: 4.63 ± 6.48 fmol/mg	0.02 pg/mg in a 50 mg sample or 5 fmol/sample	Not provided
				NNAL in smokers (17/17)	Mean ± SD: 2.58 ± 4.16 fmol/mg		
				NNN in non-smokers (12/12)	Mean ± SD: 0.35 ± 0.16 fmol/mg		
				Nicotine in non-smokers (12/12)	Mean ± SD: 0.76 ± 0.22 pmol/mg		
				Cotinine in non-smokers (12/12)	Mean ± SD: 0.044 ± 0.020 pmol/mg		
[54]	Smokers and non-smokers	20–30	Toenail clippings	Nicotine in smokers (14/14)	Mean ± SD: 1.971 ± 0.818 ng/mg	0.02 ng/mg	0.06 ng/mg
				Nicotine in non-smokers (8/10)	Mean ± SD: 0.132 ± 0.082 ng/mg		
				Cotinine in smokers (14/14)	Mean ± SD: 1.237 ± 0.853 ng/mg	0.01 ng/mg	0.03 ng/mg
				Cotinine in non-smokers (0/10)	ND		
				Myosmine in smokers (14/14)	Mean ± SD: 0.0659 ± 0.0564 ng/mg	0.035 ng/mg	0.105 ng/mg
				Myosmine in non-smokers (9/10)	Mean ± SD: 0.0212 ± 0.0149 ng/mg		
[56]	Smokers and non-smokers	≥20	Toenail clippings	Nicotine in smokers (34/34)	Median: 4.85 ng/mg	Not provided	0.005 ng/mg
				Nicotine in ETS (22/22)	Median: 0.09 ng/mg		
				Nicotine in non- smokers (4/4)	Median: 0.06 ng/mg		
Sedative and antipsychotic drugs							
[59]	Subjects on prescription medicine for sedatives	Not mentioned	Separate finger- and toenail clippings	Alprazolam (2/2)	~30 to 500 pg/mg	0.06 pg/mg	Not provided
				Clobazam (2/2),	~1000 pg/mg	0.06 pg/mg	
				Clonazepam (0/2)	ND	0.12 pg/mg	
				Diazepam (5/5)	~10 to 400 pg/mg	0.12 pg/mg	
				Lorazepam (2/2)	~5 to 60 pg/mg	0.12 pg/mg	
				Midazolam (1/1)	≲LOD to 0.2 pg/mg	0.01 pg/mg	
				Oxazepam (2/3)	~100 to 8000 pg/mg	0.60 pg/mg	
				Temazepam (1/1)	~200 to 3000 pg/mg	6.0 pg/mg	
				Triazolam (5/5)	~2 to 80 pg/mg	0.06 pg/mg	
				Zopiclone (5/5)	≲LOD to 10.1 pg/mg	0.02 pg/mg	
[11]	Volunteers ingesting 10 mg zolpidem	25	Separate finger- and toenail clippings	Zolpidem F (7/7)	<LOQ to 1.74 pg/mg	0.03 pg/mg	0.05 pg/mg
				Zolpidem T (4/4)	0.05 to 3.29 pg/mg		

Table 2 continued

Reference	Population	Sample size (mg)	Sample type	Substances detected (positive samples/total samples)	Concentration range in (positive) samples	LOD	LOQ
[12]	Volunteers ingesting 10 mg zolpidem	5–10	Fingernail clippings	Zolpidem (9/9)	0.15 to 15.1 pg/mg	Not provided	0.1 pg/mg
[60]	Patients on long-term clozapine	5	Fingernail clippings	Clozapine (16/16)	1.60 to 14.14 ng/mg	0.05 ng/mg	0.5 ng/mg
[61]	Bloated cadaver	5	Separate finger- and toenail clippings	Clozapine F (1/1) Clozapine T (1/1)	89.0 to 538.5 pg/mg 64.6 to 130.1 pg/mg	Not provided	10 pg/mg
Steroids							
[62]	7 healthy M, 9 healthy W	100	Separate finger- and toenail clippings	Testosterone F (16/16)	M: 2.05 to 5.80 ng/g; W: 0.24 to 1.69 ng/g	0.1 ng/g	0.2 ng/g
				Testosterone T (16/16)	M: 0.45 to 3.11 ng/g; W: 0.87 to 2.98 ng/g		
				Pregnenolone F (16/16)	M: 2.11 to 4.21 ng/g; W: 0.48 to 1.53 ng/g	0.1 ng/g	0.3 ng/g
				Pregnenolone T (16/16)	M: 0.30 to 3.41 ng/g; W: 1.06 to 4.33 ng/g		
[63]	Volunteers taking steroids, volunteers without steroid use	Not mentioned	Not mentioned	Testosterone (all) Testosterone propionate (none) Stanazol (1)	No quantification	Not determined	Not determined

CHD coronary heart disease, *ETS* second-hand cigarette exposure ("passive smoking"), *F* fingers, *M* men, *ND* not detected, *SD* standard deviation, *SE* standard error, *T* toes, *W* women. For other abbreviations, see the footnote of Table 1

26–32]. Most often, methanol has been employed as decontamination solvent. Two methanol washes were sufficient to remove over 98 % of the external contamination, with the exception of samples with very high cocaine levels where three methanol washes were needed [28]. Fingernail washes contained higher cocaine concentrations than the corresponding toenail washes, thereby indicating a higher degree of external contamination in fingernails [13, 28]. For both finger- and toenails, the concentrations in the washes were less than in the nails themself. In contrast, after a decontamination procedure consisting of washing with isopropanol for 15 min and phosphate buffer for 30 min, higher concentrations were present in the washes than in the nail scrapings [10]. Concentrations in the nail scrapings were 5–40 % of those in the washes, and in many samples compounds were only detected in the washes and not in the scrapings. Thus, it is likely that this decontamination procedure not only eliminated external contamination, but also removed cocaine incorporated into the nail matrix [10]. The possible degradation, mainly by hydrolysis, of cocaine analytes during extraction from the nail matrix was evaluated in several studies. Cocaine hydrolysis to benzoylecgonine was less than 5 % using Ropero-Miller's method as well as using the method presented by Garside et al. [29], and other degradation was not significant. Engelhart et al. [28] reported less than 3 % cocaine loss through the use of a phosphate buffer at pH 5 at which no hydrolysis of cocaine to benzoylecgonine occurs. However, this statement was not confirmed in a comparison study performed by Valente-Campos et al. [31]. In that study, the extraction of cocaine by methanol addition and heating at 40 °C under reflux for 16 h [33] was compared to the extraction by ultrasonic bathing with phosphate buffer for 1 h, followed by soaking for 72 h at room temperature [28]. As can be expected from the longer incubation time, hydrolysis occurred during phosphate buffer extraction, while no benzoylecgonine was detected after methanol extraction. Further investigation of the extraction recoveries showed that losses after methanol extraction were acceptable, as recoveries were above 67 % for all compounds [31].

Cocaine detection and quantification in nails

Finger- and toenail clippings from a 3-month old infant who died of sudden infant death syndrome (SIDS) were combined to collect a sufficient amount of clippings (10.1 mg) for analysis [27]. Cocaine was detected at a concentration of about 0.3 ng/mg, while its main metabolite benzoylecgonine could not be detected. This finding suggests that a higher concentration of the parent compound cocaine as compared to its metabolite benzoylecgonine (similar to hair: ratio 3:1–10:1) is deposited in the nail matrix [27]. The presence of cocaine, benzoylecgonine, norcocaine, and cocaethylene in toenails of 46 cadavers was compared to that in blood, urine, and gastric fluid [28]. Twenty-three cases were positive for cocaine and/or benzoylecgonine in toenails with concentrations ranging from 0.20 to 140 ng/mg and 0.30 to 315 ng/mg, respectively, whereas norcocaine and cocaethylene were present in only two cases [28]. Compared to other metabolites, benzoylecgonine was detected more frequently and at higher concentrations, indicating benzoylecgonine as the primary metabolite that accumulates in nails [28]. This finding was replicated and supported by another study [29] on finger- and toenail samples from 18 deceased cocaine users showing higher benzoylecgonine concentrations compared to the other cocaine metabolites (norcocaine, norbenzoylecgonine, cocaethylene, anhydroecgonine methyl ester, ecgonine methyl ester, and ecgonine ethyl ester). Cocaine was detected in nails from 14 cases (82 %) (between <0.10 and 16.1 ng/mg), while conventional toxicological screening of blood and urine only detected cocaine use in five cases (28 %), thereby indicating the difference in detection window of the matrices. Blood and urine reflect recent cocaine use, while nails indicate past or frequent cocaine exposure [28, 29]. Anhydroecgonine methyl ester, a pyrolysis product of cocaine that is a proof of cocaine use as crack [34], was detected in eight nail samples (ranging from <0.10 to >10 ng/mg) [29], thus providing evidence of different cocaine administration profiles. This application has also been documented in a murder trial, where anhydroecgonine methyl ester was found at concentrations of 0.24 and 0.39 ng/mg in nail clippings of two males suspected of murder, and resulted in the revealing of past crack consumption [30]. During a controlled dosing study, low-dose (75 mg/70 kg) and high-dose (150 mg/70 kg) cocaine was injected on three different days in eight volunteers, and fingernail scrapings were collected weekly for a period of 10 weeks [10]. Maximum cocaine concentrations of total drug detected (decontamination washes and nail specimen) ranged from 0.25 to 1.60 ng/mg (low-dose) and from 0.57 to 2.70 ng/mg (high-dose). Also, a dose–response relationship was present [10]; however, the authors acknowledged that the results may have been influenced by the method of nail collection (scraping off the nail surface). In 2002, Engelhart and Jenkins [13] analyzed finger- and toenail clippings of 15 subjects found positive by toxicological screening in blood or urine; cocaine concentrations ranged from 6.0 to 414 ng/mg in fingernails and from 1.2 to 19.9 ng/mg in toenails, but no correlation between blood and nail cocaine concentrations was observed. In the big toenails of 18 cocaine or heroin abuser cadavers, cocaine concentrations were reported in seven cases, ranging between 0.10 and 4.60 ng/mg, though without information

regarding the amount cocaine used [26]. Valente-Campos et al. [31] applied their method to nail clippings of eight cocaine users, detecting cocaine and norcocaine in only one and two samples, respectively, and benzoylecgonine in five samples (concentrations not provided); the analyzed segments of the three negative samples corresponded to abstinence or drug use less than two times a week. In nails collected from infants abandoned at birth during the first 3 months of life, cocaine and benzoylecgonine were observed in six out of 25 cases (0.14–0.25 ng/mg and 0.12–0.20 ng/mg, respectively), showing that nails from newborns can be of significant importance to determine in utero drug exposure [32].

Discussion

Similar to amphetamines, cocaine is usually present at higher concentrations than its metabolite benzoylecgonine in nails (ratio from 10:1 to 2:1) [29]. In contrast to amphetamines, cocaine is present in seven- to 20-fold higher concentrations in finger- compared to toenails [13, 29]. Aside from the difference in growth velocity between finger- and toenails, this may be due to the higher probability of external contamination with cocaine as compared to amphetamine; cocaine is manipulated and ingested as a powder, while amphetamines are usually ingested as tablets. As can be retrieved from the discussion on the pre-analytical techniques, improvements in decontamination and extraction methods are recommended. An adequate washing procedure should remove external contamination, but should not remove (part of) the incorporated cocaine or metabolites. More controlled studies should investigate this issue, which is crucial for substances often consumed as powders, as is the case for cocaine. One proposal is the use of Raman spectroscopy to visualize the presence of cocaine hydrochloride upon the human nail [35]. Finally, the detection of some specific cocaine metabolites can give additional information about concomitant use and active drug consumption. In particular, the detection of cocaethylene may indicate concomitant use of ethanol and cocaine. The detection of anhydroecgonine methylester in fingernails may suggest exposure to the smoke of crack cocaine, whereas the same finding in toenails provides more evidence in favor of the active consumption of crack. The presence of metabolic markers, such as norcocaine and norbenzoylecgonine, strongly supports cocaine ingestion [29].

Opioids

Pre-analytical and analytical techniques

Only three studies focused primarily on the detection of opiates in nails using LC coupled to an electrochemical detector (ECD), GC–MS, or LC–MS–MS [36–38]. Other studies detected opiates together with cocaine, thus using the same pre-analytical and analytical techniques as described earlier [10, 13, 26, 28, 31]. Lemos et al. [36, 37] proposed a decontamination procedure consisting of sonication in sodium dodecyl sulfate (SDS), followed by sonication in water (three washes) and methanol (three to four washes). This procedure has proved to remove any superficial contamination (i.e., radioimmunoassay screenings of the final methanol washes were negative for morphine/methadone). Hydrolysis of 6-monoacetylmorphine (6-MAM) was less than 10 % following the sample preparation procedure proposed by Ropero-Miller [10]. To avoid the hydrolysis of 6-MAM to morphine in alkaline and acidic conditions, Shen et al. [38] proposed an alternative extraction method consisting of incubation of the nail samples in a borate buffer (pH 9.2) for 30 min. In addition, frozen pulverization was applied to increase the specific surface area of the samples which influences the extraction, thereby improving the sensitivity of the detection method (LOQ = 0.05 ng/mg) [38].

Opioid detection and quantification in nails

Morphine and 6-MAM, two active metabolites of heroin, as well as codeine and hydrocodone were analyzed in toenails of 34 cocaine users described earlier by Engelhart et al. [28]. However, only a few samples tested positive for opioids. Three cases showed the presence of morphine and 6-MAM (range 0.16–0.72 ng/mg and 0.41–1.70 ng/mg, respectively), two of which were positive for codeine (1.02 and 3.07 ng/mg) and one for hydrocodone (0.62 ng/mg) [28]. Codeine was detected in nail scrapings from eight individuals who received controlled oral doses of codeine sulfate on three different days. Low-dose codeine sulfate (60 mg/70 kg) resulted in maximum codeine concentrations of total drug detected (decontamination washes and nail specimen) from 0.12 to 0.31 ng/mg detected in four cases, while high doses (120 mg/70 kg) resulted in maximum codeine concentrations of total drug detected from 0.12–0.20 ng/mg detected in six cases [10]. This suggests that although a single use of codeine might not be detected, regular (chronic) use can be. In 22 out of 26 nail samples obtained from treatment-seeking heroin users, morphine was detected at concentrations from 0.14 to 6.90 ng/mg, but with variation in morphine levels among individuals who declared to have consumed the same amounts of heroin (based on the amount of money spent for heroin) [37]. From the four samples where morphine was not detected, two had very low sample weights (3 and 6.3 mg), which may have contributed to the negative results found in these samples [37]. Lemos et al. [36] detected methadone (between 0.51 and 363 ng/mg) in

fingernail clippings of 27 out of 29 individuals attending a methadone-maintenance clinic. In both studies, the authors ascribed the absence of a (meaningful) dose–response relationship to the relatively small sample sizes ($n = 26$ and 29, respectively), the heterogeneity of street heroin, the differences in consumption patterns, the unclear mechanisms of drug incorporation into nails, and the unreliability of self-reports [36, 37]. Nevertheless, according to Lemos et al. [36], future studies should investigate the use of nail analysis for the purpose of monitoring compliance to, e.g., methadone-maintenance programs. Finger- and toenails from 17 postmortem cases were analyzed for a variety of opioids. Morphine, 6-MAM, and codeine were detected in most cases: morphine in 15 cases from 0.05 to 408 ng/mg, 6-MAM in 15 cases from 0.04 to 504 ng/mg, and codeine in nine cases from 0.06 to 8.84 ng/mg. In contrast, hydromorphone, oxycodone, and hydrocodone could only be detected in a few subjects: hydromorphone in four cases from 0.12 to 0.45 ng/mg, oxycodone in one case from 5.05 to 6.88 ng/mg, and hydrocodone in one case (result not mentioned) [13]. Cingolani et al. [26] showed that morphine was more concentrated in toenails (mean concentration 1.27 ng/mg) as compared to hair (mean concentration 0.79 ng/mg), but that this was not the case for 6-MAM (mean concentration 0.46 ng/mg in toenails vs. 0.50 ng/mg in hair). However, a direct comparison with fingernails is lacking so far. In nail clippings from 25 newborns abandoned after birth, morphine (in four cases; 0.09–0.15 ng/mg) and methadone (in five cases; 0.12–0.26 ng/mg) were detected, providing a first indication of in utero opioid exposure [32]. In the same study, methadone was detected in two out of 33 nail samples from newborns at similar concentrations (0.16 and 0.17 ng/mg). In fingernail clippings from 18 subjects whose urine tested positive for morphine, morphine, 6-MAM, and codeine were positive in 12 cases (ranges 0.58–3.16 ng/mg, 0.10–1.37 ng/mg and <0.05–0.27 ng/mg, respectively), whereas acetylcodeine and heroin were only detectable in one and three subjects, respectively (concentrations < LOQ) [38].

Discussion

Opioid and cocaine detection are often associated [10, 13, 26, 28, 31], because both alkaloids are often co-consumed to enhance the effects (i.e., "speed balling"). Two studies which focused exclusively on the detection of opioids in nails from documented opioid abusers indicate that nail analysis can be used to detect and quantify opioids, and to determine treatment compliance [36, 37]. In addition, these studies tried to establish a dose–response relationship by comparing the concentrations in nails to the dose of heroin [37] and the prescribed dose of methadone [36]. However, more research by means of dose-controlled studies is needed to establish dose–response relationships and to know whether nail analysis can provide cumulative drug use information.

Cannabinoids

Pre-analytical and analytical techniques

Detection of Δ^9-tetrahydrocannabinol (THC) and its metabolite 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) has been performed using GC–EI–MS as well as using GC–MS–MS operated in negative ion chemical ionization (NICI) mode [23, 39, 40]. Using GC–NICI–MS–MS, a high sensitivity was achieved with an LOD of 0.01 pg/mg [39] as compared to LODs between 35 and 100 pg/mg obtained by GC–EI–MS [23, 40]. As for opioids, washes with SDS, water and methanol both in triplicate were effective in removing the superficial contamination because the third methanol wash produced negative cannabinoid results [40]. However, a decontamination procedure without the SDS washing step was proved to be effective as well (no substances were detected in a methanol wash performed after the normal washing procedure) [23]. In all studies, alkaline digestion was used as the extraction procedure. When performing LLE for further clean-up, the influence of the pH needs to be considered. In contrast to THC, THC-COOH could only be detected in the nail hydrolysates extracted under acidic pH and not in hydrolysates extracted under alkaline conditions [40].

Cannabinoid detection and quantification in nails

Together with a brief questionnaire to assess the use of cannabis and other drugs, fingernail clippings from 23 cannabis users were sampled and analyzed [40]. Of these, 12 subjects provided weekly THC use estimates, and THC was detected in 11 of 14 tested samples at concentrations varying from 0.13 to 6.97 ng/mg, and detectable up to 9 months after sample collection [40]. THC-COOH was detected in two out of three nail hydrolysates extracted under acidic pH (9.82 and 29.7 ng/mg) [40]. In nine suspected drug users, THC-COOH was detected in only one sample (0.20 ng/mg), whereas THC could not be detected [23]. Mean THC-COOH concentrations in fingernails of 60 students were on average five times higher than mean THC-COOH concentrations in hair samples from the same individuals (1.8 pg/mg and 0.4 pg/mg, respectively), suggesting a higher incorporation of THC-COOH in nails [39]. Furthermore, due to the higher concentrations present in nails, THC-COOH was detectable in a higher percentage of nail samples (53 %) as compared to that of hair samples (47 %) [39].

Discussion

So far, only three studies detected THC and/or its metabolite THC-COOH in nails [23, 39, 40]. The detection of THC-COOH, which is only formed through THC metabolism, is important to differentiate between active cannabis consumption and external contamination through cannabis smoke. Because concentrations of THC-COOH were higher in nails than in hair, Jones et al. [39] proposed nails as the preferred matrix for the detection of cannabinoids. The concentrations of THC-COOH reported by Jones et al. were much lower (range <0.01–0.052 pg/mg) than those reported in the previous studies (range 0.20–29.7 ng/mg) [23, 40]. A possible explanation is that the subjects consumed less cannabis; however, accurate self-reports were absent. Because only a few studies are available and very low concentrations have to be detected (especially for THC-COOH), more research as well as more sensitive methods are required to evaluate the use of nails for the detection of cannabinoids.

Ethyl glucuronide

Pre-analytical and analytical techniques

Available methods for the analysis of ethyl glucuronide (EtG), a minor metabolite of alcohol, in nails all employed LC–MS–MS with electrospray ionization (ESI), but used different sample preparation procedures [41–45]. Using an SPE procedure for the sample preparation, Morini et al. [42] achieved low LODs and LOQs (2 and 8 pg/mg, respectively), and improved the sensitivity of the analytical method.

Ethyl glucuronide detection and quantification in nails

Morini et al. [42] reported that EtG concentrations in fingernails were higher than those in hair and correlated with self-reported alcohol consumption. Ethyl glucuronide was detected in the fingernails of five individuals consuming >10 g alcohol per day, with concentrations ranging from 12.3 pg/mg (for an individual consuming 10–30 g/day) to 92.6 pg/mg (for an individual consuming >60 g/day). When investigating the correlation between alcohol intake and EtG concentrations in fingernails a linear correlation was observed ($r = 0.801$; $P < 0.001$), providing evidence that nails can be useful to assess alcohol intake behavior [44]. In fingernail samples from 529 students, EtG was detected in 38 % of the samples (203 samples) with values ranging from <2 to 397 pg/mg [41, 45]. Upon assessment of the alcohol consumption of 447 college students during 12 weeks, Berger et al. [45] proposed the following cut-offs: 8 pg/mg to detect any (>0) alcohol consumption per week, 37 pg/mg to detect >15 drinks per week and 56 pg/mg to detect >30 drinks per week. Nails and hair of 18 mothers, whose child's meconium was found to be positive for fetal ethanol biomarkers, were all negative for EtG [43]. These results suggest that maternal nails, just as maternal hair, are not suitable to disclose alcohol consumption lower than 15–30 g/day.

Discussion

In summary, the detection of EtG in nails is a specific and sensitive biomarker for the detection of alcohol use, with a sensitivity higher than hair (due to a higher degree of accumulation) [42]. Still, no studies were performed in toenails and the available methods have relatively high LOQs to enable unequivocal quantification of EtG at the proposed cut-off value of 8 pg/mg [45]. More sensitive techniques (i.e., with lower LOQs) might be obtained using GC–MS–MS, similar to methods for EtG quantification in hair (for a review, see [46]). Because of higher EtG levels present in nails as compared to hair, nails may potentially be the preferred specimen to differentiate teetotalers from moderate alcohol consumers. Furthermore, Morini et al. [42] suggested that, because of the higher accumulation of EtG in nails, nail analysis may allow the accurate evaluation of binge drinking behavior.

Ketamine and phencyclidine

Pre-analytical and analytical techniques

Ketamine and norketamine can be detected using methods presented earlier for amphetamines [24, 25]. Phencyclidine (PCP) was detected using GC–EI–MS after alkaline digestion of a large amount of nails (100–200 mg) [47], followed by LLE previously developed for citalopram detection [48]. No information on LOQ or LOD in nails was provided [47].

Ketamine and phencyclidine detection and quantification in nails

Only two studies reported the detection of (nor-)ketamine in nails [24, 25], both in combination with the detection of amphetamines. In fingernail clippings from seven multi-drug users, ketamine and norketamine were detected in only one case at concentrations below the LOQs at 0.314 and 0.050 ng/mg, respectively [24]. Toenail samples obtained from four drug users all tested negative for norketamine [25]. PCP was reported in nail samples collected during autopsy from four drug abusers, whose blood

or urine tested positive for PCP [47]. PCP concentrations in nails ranged from 0.33 to 148 ng/mg, and higher concentrations were found in fingernails as compared to toenails. Nail PCP concentrations were not correlated with blood PCP concentrations [47].

Discussion

Except for one report on the presence of (nor-)ketamine in nails and one report on the detection of PCP in four cases, no studies in documented ketamine or PCP users are available, and the research is highly recommended.

Caffeine, nicotine, and cotinine

Pre-analytical and analytical techniques

LC–ECD or GC–MS were used for the detection of nicotine, cotinine, and caffeine in toenails [32, 49–56]. To allow the detection of the tobacco-specific *N*-nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and *N*-nitrosonornicotine (NNN) in toenails, which are only present at very low concentrations, a more sensitive technique using LC–ESI–MS–MS was developed [51, 57]. The sample preparation procedure was similar between studies, and included washing with dichloromethane, digestion with sodium hydroxide and LLE followed by LC–ECD and GC–MS analyses [49–55] or SPE followed by LC–ESI–MS–MS analyses [51, 57]. No information about the evaluation of the pre-analytical procedure is available.

Caffeine, nicotine, and cotinine detection and quantification in nails

Toenail nicotine levels were investigated as a biomarker for active and passive tobacco smoke exposure by comparison to self-reported smoke exposure. Mean toenail nicotine concentrations in a large cohort study ($n = 1{,}696$) were 0.10, 0.14, 1.77 ng/mg for non-smokers, passive smokers, and active smokers, respectively [52], and were in agreement with previous results in a smaller population ($n = 104$) [49]. Although toenail nicotine concentrations were correlated with reported smoking exposure categories, there was no complete concordance between both. This suggests that measurement of nicotine concentrations in nails reveals sources of exposure that are not captured from standard questionnaires. The potential of toenail nicotine levels to estimate associations with health risks was investigated for coronary heart disease and lung cancer [53, 55]. In both studies a dose–response relation between nail nicotine concentrations and health risks was observed. Cotinine, NNAL, and NNN in toenails were first reported by the research group of Stepanov et al. [50, 51, 57]. NNAL and NNN, tobacco-specific *N*-nitrosamines, are of particular concern due to their carcinogenic potency [58]. Nicotine, cotinine, NNAL, and NNN in toenails were validated as biomarkers of tobacco smoke exposure by investigation of their correlation with other biomarkers for smoking such as plasma nicotine, cotinine and trans-3′-hydroxycotinine, and urinary NNAL [51]. Schutte-Borkovec et al. [54] found smoking-dependent differences for the tobacco alkaloid 3-(1-pyrroline-2-yl)pyridine or myosmine in toenails, but these were much smaller than those found for nicotine and cotinine. These results indicate that factors other than tobacco contribute to the burden of myosmine and that this compound is not as specific for smoke exposure as nicotine and cotinine are [54]. By investigation of nicotine in nails, Hsieh et al. [56] found that smoking history underestimates the prevalence of active and passive smoking exposure; this underlines the importance of reliable biomarkers of smoke exposure for the estimation of associated health risks. Caffeine, nicotine, and cotinine were detected in nail samples from two groups of newborns collected after birth (concentrations not provided) [32]. Caffeine was detected in six out of 33 newborns and corresponded to mother self-reports of caffeine consumption during pregnancy. In six out of 33 cases nicotine and/or cotinine were found in nails of newborns from non-smoking mothers. These cases could indicate passive nicotine inhalation or failure to admit cigarette use owing to feelings of guilt [32].

Discussion

Tobacco alkaloids, including nicotine, cotinine, NNAL, and NNN, can be detected in toenails and are suitable long-term biomarkers for active and passive smoke exposure. As a result of their slow growth rate, nails can reflect cumulative exposure over a relatively long period, thereby overcoming both the subjectivity of self-reported questionnaires and the day-to-day exposure variation [49, 52]. Moreover, toenail nicotine levels provide additional information on active and passive smoke exposure not captured by reported history, and is a good predictor of coronary heart disease and lung cancer risk [50, 51, 53, 55, 57]. In the assessment of smoke exposure, toenails are preferred over fingernails as they are relatively free from external contamination. However, no studies reported fingernail nicotine concentrations. Hence, a comparison between toe- and fingernails should be performed to provide evidence for this statement. Data on caffeine detection in nails are very scarce. Only one study in a population of newborns reported caffeine in nails [32].

Sedative and antipsychotic drugs

Pre-analytical and analytical techniques

LC–ESI–MS–MS is the favored method for the detection of a variety of sedatives and the antipsychotic clozapine [11, 12, 59–61]. Interestingly, Madry et al. [12] developed a method without any washing step; they assumed that daily hygiene was sufficient for decontamination, and additional washing procedures may lead to unwanted extraction effects. All studies included a homogenization step in which nails were cut or pulverized. LODs and LOQs were in the pg/mg range, except for one method with a relatively high LOD and LOQ (0.05 and 0.5 ng/mg, respectively) [60].

Sedative and antipsychotic drug detection and quantification in nails

Finger- and/or toenail clippings obtained from 21 subjects who were prescribed sedatives were screened for the presence of alprazolam, clobazam, clonazepam, diazepam, lorazepam, midazolam, oxazepam, temazepam, triazolam, zopiclone, and selected metabolites [59]. With the exception of clonazepam, all screened sedatives were detected in nails, with higher concentrations in nails of subjects taking higher sedative doses [59]. Chen et al. [60] reported the detection of clozapine and its major metabolite nor-clozapine in fingernail clippings of 16 volunteers who were prescribed clozapine for more than 9 months (range 1.60–14.1 ng/mg). The obtained nail clippings were sampled about 10 years ago providing first evidence for the long-term and stable storage of antipsychotics in nails [60]. This long-term stability was further confirmed by the detection of clozapine in finger- and toenails from a bloated cadaver (range 64.6–539 pg/mg) [61]. Two studies investigated the incorporation mechanisms of zolpidem in nails after a single dose [11, 12]. In the former study, finger- and toenail clippings from seven subjects receiving a 10-mg dose of zolpidem were collected weekly, every 2 or 4 weeks for 20 weeks [11]. In the long-term follow-up analysis, two peaks of relatively high zolpidem concentrations were identified. The initial high levels (between 0.40 and 1.74 pg/mg in fingernails) were observed in the first week after consumption (probably resulting from incorporation through sweat). Lower peak concentrations (<0.37 pg/mg in fingernails) were observed between 10 and 15 weeks after intake (from the germinal matrix). Between the first and second concentration peaks an interval with lower zolpidem levels was observed (from the nail bed). Overall, toenails showed higher concentrations as compared to fingernails [11]. In the latter study, fingernail clippings from nine subjects who received a 10-mg zolpidem dose were collected weekly during 3 to 5 months [12]. The results from the concentration–time curve were as follows: a high initial concentration (0.8–15.1 pg/mg) 24 h after intake (presumably caused by sweat-mediated transport), a concentration peak (0.15–2.2 pg/mg) after 2–3 weeks (through incorporation via the nail bed), and a concentration peak (0.15–0.9 pg/mg) after 10–18 weeks (through incorporation via the germinal matrix). The median window of detection was 13.5 weeks (standard deviation = 24 %) [12].

Discussion

The detection of sedative and antipsychotic drugs in nails is relatively recent. Studies indicate that nails can be useful for the detection of sedatives and antipsychotics [11, 12, 59–61]. Nail samples from individuals taking higher sedative doses contained higher concentrations of sedatives [59], showing an accumulation of sedatives in nails upon frequent use. Clozapine detection in samples collected 10 years ago and in samples of a bloated cadaver provided evidence for the stable storage of xenobiotics in nails and indicated that nails could be useful in postmortem forensic toxicology [60, 61]. The studies on the incorporation mechanisms in nails suggest that drugs incorporate into nails from three different sources: sweat, nail bed, and nail matrix [11, 12]. In addition, those studies showed that even a single exposure to zolpidem could be detected in nails, which offers major possibilities for application in forensic toxicology. Therefore, research should be extended towards the detection of a single exposure to other drugs of abuse or pharmaceuticals and their detection window.

Steroids

Pre-analytical and analytical techniques

Two studies reported the detection of steroids in nails using different techniques. Choi et al. [62] employed GC–EI–MS, while Brown and Perrett [63] used LC combined with ultraviolet (UV) detection as the first instance. However, as LC-UV was found unsuitable for the detection of steroids in nails, the authors switched to LC–ESI–ion-trap–MS [63]. Choi et al. [62] used a relatively high amount of sample (100 mg) and a sample preparation procedure consisting of washing with methanol, alkaline digestion, followed by LLE, and pentafluorophenyl dimethylsilyl-trimethylsilyl (flophemesyl-TMS) derivatization. Because no information on sample preparation except for the use of extraction is provided by Brown and Perrett, a comparison between sample preparation procedures of both studies is not possible [62, 63].

Steroid detection and quantification in nails

Pregnenolone and testosterone were detected in finger- and toenails of seven healthy men and nine healthy women in concentration ranges of 0.30–4.33 ng/g and 0.24–5.80 ng/g, respectively [62]. Concentrations of both steroids were higher in males than in females. This gender difference may reflect a correlation between steroid concentrations in serum (higher in males than in females) and nails [62]. Brown and Perrett collected nail samples from volunteers who had been taking anabolic–androgenic steroids over the last 6 months, and from volunteers without any history of steroid use [63]. Testosterone could be detected in all samples and stanazol in one sample, while testosterone propionate could not be detected in any sample. Because the method sensitivity did not meet acceptable standards for quantification, the authors were only able to qualitatively show the presence of steroids in nails.

Discussion

Although two studies reported the detection of steroids in nails, only the method developed by Choi et al. [62] allowed the quantification of these compounds. In addition, only three different steroids were investigated so far [62, 63]. Steroid analysis in nails could be applied to detect steroid (ab)use in athletes, and awaits further investigation.

Antimycotics

Several studies reported on the detection of antimycotic agents, such as itraconazole, fluconazole, and terbinafine, in nails to retrieve information on the correlation between nail concentrations and antifungal therapeutic efficiency. These studies are extensively described in a review by Palmeri et al. [1]. Since then, no new studies were published.

Simultaneous detection of multiple drugs

Analytical methods for multiple drug detection were reported. There are reports on the simultaneous detection of amphetamines and ketamine [24, 25], opiates and cocaine [10, 13, 26–28, 31, 32], and various sedatives [59]. One study proposes the combined detection of amphetamines and THC [23]. The challenge of these methods lies in the optimization of the (pre-)analytical techniques to allow the detection of multiple compounds with different physicochemical characteristics.

Nails as a specimen for drug screening

Recently, a first study investigated the potential use of nails for general unknown screening (GUS) [64]. Using LC–quadrupole time-of-flight (QTOF)–MS, 89 different compounds were detected in nail samples from 70 postmortem cases. The results indicated that GUS in nails could be useful for the detection of long-term drug consumption, especially in cases where no information on the subject or ingested substances is available.

Applications of nail analysis

Identification of in utero drug exposure

Alcohol and drug use during pregnancy can lead to miscarriage, premature birth, increased mortality, congenital abnormalities, and retarded physical and mental development [65, 66]. Newborn nails are formed during the second trimester of pregnancy [67], grow continuously, and persist after birth, thus providing an opportunity to assess in utero drug exposure. In contrast, neonate hair growth starts during the third trimester, occurs in cycles and hair is generally lost within 8–12 weeks after birth [68]. In a 3-month-old infant who died of SIDS, the presence of cocaine in finger- and toenails was detected and correlated to intra-uterine exposure [27]. A study in 58 newborns confirmed the usefulness of neonatal nails for detecting in utero drug exposure to cocaine, opioids, caffeine, nicotine, and cotinine [32]. Conversely, another study suggests that, in contrast to neonatal meconium, maternal nail clippings could not be used to assess in utero drug exposure to alcohol less than daily use (<15–30 g alcohol/day) [43]. Indeed, in 18 cases, in which EtG and fatty acid ethyl esters in meconium were found to be positive, none of these cases could be confirmed by the presence of EtG in maternal nails. However, this neither excludes the use of maternal nails to detect higher alcohol consumption, nor the use of neonatal nails to detect maternal alcohol consumption.

Monitoring of drug-treatment programs

Alcohol and drug abuse have serious negative consequences on the individual and the society. In drug-dependence treatment settings, nail analysis can be useful for the monitoring of patients. In addition, it could be used to identify objectively patients who relapse during treatment and may need additional treatment. One example is monitoring of methadone maintenance programs. In patients following such a program, nail analysis showed to be a useful tool to assess compliance to the treatment scheme

[37]. However, the use of nails to monitor abstinence from alcohol and/or drugs of abuse within a treatment program has not been documented yet.

Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) in nails has been described within the context of onychomycoses in order to assess the relationship between antimycotic concentration at the site of action and therapeutic outcome (for a review, see [1]). Although nail analysis cannot be used for dose titration and real-time TDM, it could be a useful tool in monitoring long-term therapy compliance of antidepressants and antipsychotics.

Forensic toxicological applications

Nails offer a substantially longer retrospective detection window as compared to body fluids, which is often vital for solving cases of death due to drug use or poisoning, or upon (a delayed) reporting of a possible rape using drug(s).

The use of nails in postmortem circumstances was first reported for cocaine [27, 28]. In postmortem toxicology, nails can (i) support death diagnosis by proving or excluding chronic substance abuse, (ii) provide evidence for drug tolerance in opioid death cases, and (iii) contribute to the identification of an unknown corpse. Consequently, it has been suggested that nails can be used to complement the other biological matrices, narrow the scope of an investigation, and provide vital clues in resolving a case [61]. In postmortem toxicology, besides the collection of nail clippings or scrapings, it is also possible to collect the entire nail plate. This offers several advantages: more nail is available for analysis, pieces close to the germinal matrix can be analyzed, and scrapings from the underside of the nail plate allow additional horizontal segmentation.

Over the last several years, the number of drug-facilitated sexual assaults (DFSA) has increased considerably [69]. Most drug-facilitated crimes involve a single exposure to alcohol, drugs of abuse, or pharmaceuticals, and upon rapid presentation of the victim, blood and urine are the preferred samples for toxicological investigation. Considering that the majority of victims wait several days before reporting to the police, nails could be used in those cases. From their study, Irving et al. [59] concluded that nails could potentially be useful for the detection of sedatives, for example in cases of suspected DFSA.

Other applications

The detection of doping agents in nails could potentially be useful to provide long-term information, and would be particularly interesting for substances prohibited both in and out of competition. Still, the zero tolerance policy for doping agents requires very sensitive methods to address the unequivocal absence of a controlled substance. Such methods are not available for nails and illustrate the necessity of more research on the matter.

Thus far, the use of nails in the context of workplace drug testing has not been described. Nevertheless, nail analysis could offer important benefits, including the detection of chronic use over a relatively long time span before sampling. This could be useful, e.g., in the context of the zero-tolerance policy for airline pilots and for professionals in rehabilitation from alcohol- or drug-dependencies.

Current pitfalls in nail analysis and challenges for future research

Factors influencing substance incorporation

Mechanisms for substance incorporation into nails have only been scarcely investigated. Thus far, only two studies [11, 12] reported on the incorporation of substances in fingernails, both after administration of a single dose of zolpidem as mentioned before. The findings indicate that incorporation into fingernails occurs (i) by sweat-mediated transport (detectable after 24 h), (ii) through the vertical growth of the nail bed (detectable after 2 weeks), and (iii) through the horizontal growth of the germinal nail matrix (detectable after 10 weeks).

Substance incorporation can be influenced by several factors, including nail-specific, individual-specific, and substance-specific characteristics (e.g., growth rate and physical state of the nail, age, and gender of the individual, physicochemical properties of the substance). For example, variations in growth rate can lead to differences in incorporation levels of ingested substances. While this effect may be minor due to the relatively slow growth rate of nails per se, it may bias the (retrospective) time frame that is interpreted. So far, not many studies have investigated the factors influencing nail growth, and the majority of such studies were published before 1980. Moreover, several authors did not examine whether the differences that they found were significant or not, and not all the influencing factors were confirmed by later studies. Table 3 summarizes the factors that have been consistently reported by most studies to affect the growth rate of nails [70–76].

Another important factor that might alter substance incorporation into nails is the use of nail polish or, more importantly, acetone when removing the nail polish. Thus far, no studies have investigated the influence of these cosmetic treatments on drug concentrations in nails.

Table 3 Overview of the factors influencing the growth rate of nails

Faster growth rate	Slower growth rate
Fingernails	Toenails
Male	Female
Young age	Older age
Summer, elevated temperatures	Winter, lowered temperatures
Pregnancy	Malnutrition
Increased blood supply	Decreased circulation
Hyperthyroidism	Hypothyroidism
Onycholysis	Acute infection (e.g., measles, mumps)
Minor trauma, onychophagia	Onychomycosis
Regeneration after avulsion	Immobilization
Epidermal hyperproliferation affecting the skin and nails (e.g., psoriasis)	
Drugs (e.g., biotin, terbinafine, itraconazole, fluconazole)	Drugs (e.g., methotrexate, azathioprine, cyclosporine)

Given the lack of a complete understanding of the mechanisms and factors influencing substance incorporation, results of nail analysis for retrospective detection of xenobiotic use should be interpreted with caution. More research is required on this topic, including studies assessing nail-specific, individual-specific, and substance-specific characteristics that may alter incorporation of substances into nails.

Handling of external contamination

In order to avoid false interpretations due to external contamination, careful decontamination of nail samples is of great importance. In addition, evaluation of drug concentrations in the wash fractions can provide evidence on the efficiency of the washing procedure. An interesting feature of nails in this context is that sampling can be performed in two ways: vertical segmentation achieved by nail clipping, or horizontal segmentation by nail scraping. Scraping off the upper nail layer can reduce external contamination, and thus, increase the reliability of the obtained results. When the entire nail is available, scraping off the underside of the nail can further eliminate external contamination. Finally, contamination from manipulation of the substance (especially important for powdered substances like cocaine) will mainly be found in fingernails, while contamination from sweat will mainly be observed in toenails. Consequently, comparison of the results obtained in finger- and toenails gives an indication about the extent and source of external contamination.

Detection of a single exposure to a drug of abuse or pharmaceuticals

Conducted studies have indicated that regular use of drugs of abuse and pharmaceuticals can be detected in nails, but few studies focus on the detection of single or intermittent use. Although it has been shown that a single administration of zolpidem [11] can be detected, it remains unknown if a single exposure to other drugs or pharmaceuticals can also be detected. Therefore, more sensitive and specific methods should be developed, and using these methods, single exposure to other compounds should be investigated in nails.

Comparison and correlation of results

The lack of standardized sampling techniques, pre-analytical, and analytical methods makes it difficult to compare the results of the conducted studies. Indeed, the relatively large variations in pre-analytical and analytical methods employed can influence the obtained results. Also, proficiency testing programs to verify the quality of the developed methods and their results, and to compare results between laboratories are not available for nail analysis. No cut-off values exist to aid interpretation. Moreover, the quality of the results in studies is hampered by the unreliability of self-reports of drug use, the limited number of paired samples and the lack of controlled dosing. For several drugs, including sedatives, EtG and cocaine, there appears to be a dose–response relationship [42, 59, 77]. However, there does not seem to be any correlation between blood concentrations and the concentrations found in nails [13]. Evaluation of the time of delay between drug intake and detection in nails has not been performed yet.

Nail analysis in comparison to hair analysis

Nails and hair are the sole matrices known to store xenobiotics over relatively large periods of time (months to years), and from which retrospective information on drug use can be retrieved. As a consequence of their keratinized nature, nails and hair have several characteristics in common, but differ from each other in some aspects. Firstly, the double incorporation mechanisms in nails (nail bed and germinal matrix) have consequences regarding the segmentation of the matrix (horizontally and vertically). Secondly, nails grow slower than hair, which allows the detection of smaller levels of exposure, because of the higher accumulation, and the investigation of longer periods of time. Thirdly, hair is characterized by a cyclic growth rate with different stages, whereas nails grow at a

constant rate, which facilitates the interpretation of results. Finally, melanin concentration in hair is known to influence the extent of incorporation depending on the physicochemical properties of drugs [78, 79]. Because nails do not contain melanin, the bias due to pigmentation is absent for nails.

As a result of these differences, a variation in the extent of incorporation between both matrices can be expected (depending on the physicochemical properties of a substance). This seems to be the case for EtG and cannabinoids which have been detected in higher concentrations in nails as compared to hair. Considering the low concentrations that have to be measured, the higher sensitivity of nails to detect EtG and cannabinoids represents a large advantage over hair analysis. Studies comparing amphetamine concentrations in hair and nails, show higher concentrations in nails on one hand [22], and lower or similar concentrations in nails as compared to hair on the other hand [8, 16, 21]. For sedatives and clozapine, hair could be preferred because higher to similar concentrations are observed in hair as compared to nails [59, 60]. Nevertheless, data on comparison of both matrices are scarce and more research is necessary to enable definitive conclusions. Data obtained from GUS of nails were comparable to those obtained from hair analysis, because only 10 % of the cases showed a disagreement of results [64].

Conclusions and further perspectives

Nail analysis is a promising tool for the long-term detection of exposure to drugs and pharmaceuticals in both forensic and clinical applications. Nail analysis can complement blood and urine analysis, and provide additional information crucial for a correct interpretation of the results. Studies show that most drugs of abuse and pharmaceuticals are detected in nails in the pg/mg to ng/mg range. Thus, nail analysis requires sensitive and specific analytical methods, as well as an optimized sample preparation procedure.

Still, more research on nails is necessary to allow a comprehensive evaluation of this matrix and to gain more experience with nail analysis. Future investigations should address the following issues. The variety of substances that have been investigated in nails is rather limited and needs to be elaborated. For example, the detection of γ-hydroxybutyric acid in nails has never been investigated, and the detection of pharmaceuticals is limited to some sedatives and clozapine. Nail analysis needs to be implemented in forensic and clinical applications, such as workplace drug testing and TDM. Mechanisms of drug incorporation into nails and factors influencing this incorporation deserve further investigation. Dose-controlled studies, in which drug intake or consumption profiles are compared with drug levels detected in nails and other matrices, should be conducted to evaluate the correlation of drug concentrations in nails with drug intake and concentrations in other biological matrices, such as blood, urine, and hair. Also, more paired-sample studies regarding the comparison of nails with hair are highly recommended. Sampling techniques, pre-analytical, and analytical methods need to be harmonized and standardized. Proficiency testing programs should be developed and cut-off values should be proposed.

In summary, this review clearly indicates the potential of nails as matrix for the detection of drug of abuse and pharmaceutical exposure over extended time periods. Currently, the major drawback of nail analysis is the lack of research, which complicates the understanding and interpretation of results obtained by nail analysis. Augmented knowledge on nails is needed to draw definitive conclusions on the significance and appropriateness of its forensic and clinical applications.

Acknowledgments PhD student Delphine Cappelle is grateful to the University of Antwerp for her scholarship. Dr. Alexander van Nuijs acknowledges the Flanders Scientific Funds for Research (FWO) for his grant. Our thanks also go to Dr. Catherine Denis who provided valuable comments to the writing of this review.

Conflict of interest There are no financial or other relations that could lead to a conflict of interest.

References

1. Palmeri A, Pichini S, Pacifici R, Zuccaro P, Lopez A (2000) Drugs in nails: physiology, pharmacokinetics and forensic toxicology. Clin Pharmacokinet 38:95–110
2. Daniel CR, Piraccini BM, Tosti A (2004) The nail and hair in forensic science. J Am Acad Dermatol 50:258–261
3. Lander H, Hodge PR, Crisp CS (1965) Arsenic in the hair and nails: its significance in acute arsenical poisoning. J Forensic Med 12:52–67
4. Pounds CA, Pearson EF, Turner TD (1979) Arsenic in fingernails. J Forensic Sci Soc 19:165–173
5. Alexiou D, Koutselinis A, Manolidis C, Boukis D, Papadatos J, Papadatos C (1980) The content of trace elements (Cu, Zn, Fe, Mg) in fingernails of children. Dermatologica 160:380–382
6. Wilhelm M, Hafner D, Lombeck I, Ohnesorge FK (1991) Monitoring of cadmium, copper, lead and zinc status in young children using toenails: comparison with scalp hair. Sci Total Environ 103:199–207
7. Gerhardsson L, Englyst V, Lundstrom NG, Nordberg G, Sandberg S, Steinvall F (1995) Lead in tissues of deceased lead smelter workers. J Trace Elem Med Biol 9:136–143
8. Suzuki O, Hattori H, Asano M (1984) Nails as useful materials for detection of methamphetamine or amphetamine abuse. Forensic Sci Int 24:9–16
9. Gupchup GV, Zatz JI (1999) Structural characteristics and permeability properties of the human nail: a review. J Cosmet Sci 50:363–385
10. Ropero-Miller JD, Goldberger BA, Cone EJ, Joseph RE Jr (2000) The disposition of cocaine and opiate analytes in hair and

fingernails of humans following cocaine and codeine administration. J Anal Toxicol 24:496–508
11. Hang C, Ping X, Min S (2013) Long-term follow-up analysis of zolpidem in fingernails after a single oral dose. Anal Bioanal Chem 405:7281–7289
12. Madry MM, Steuer AE, Binz TM, Baumgartner MR, Kraemer T (2014) Systematic investigation of the incorporation mechanisms of zolpidem in fingernails. Drug Testing and Anal 6:533–541
13. Engelhart DA, Jenkins AJ (2002) Detection of cocaine analytes and opiates in nails from postmortem cases. J Anal Toxicol 26:489–492
14. Marieb EN (2012) Essentials of human anatomy and physiology, 10th edn. Pearson, New Jersey
15. Fleckman P (1985) Anatomy and physiology of the nail. Dermatol Clin 3:373–381
16. Lin D-L, Yin R-M, Liu H-C, Wang C-Y, Liu RH (2004) Deposition characteristics of methamphetamine and amphetamine in fingernail clippings and hair sections. J Anal Toxicol 28:411–417
17. de la Torre R, Farre M, Navarro M, Pacifici R, Zuccaro P, Pichini S (2004) Clinical pharmacokinetics of amfetamine and related substances: monitoring in conventional and non-conventional matrices. Clin Pharmacokinet 43:157–185
18. Pragst F, Balikova MA (2006) State of the art in hair analysis for detection of drug and alcohol abuse. Clin Chim Acta 370:17–49
19. Kronstrand R, Förstberg-Peterson S, KÅgedal B, Ahlner J, Larson G (1999) Codeine concentration in hair after oral administration is dependent on melanin content. Clin Chem 45:1485–1494
20. Testorf MF, Kronstrand R, Svensson SPS, Lundström I, Ahlner J (2001) Characterization of [^{3}H]flunitrazepam binding to melanin. Anal Biochem 298:259–264
21. Suzuki S, Inoue T, Hori H, Inayama S (1989) Analysis of methamphetamine in hair, nail, sweat, and saliva by mass fragmentography. J Anal Toxicol 13:176–178
22. Cirimele V, Kintz P, Mangin P (1995) Detection of amphetamines in fingernails: an alternative to hair analysis. Arch Toxicol 70:68–69
23. Kim JY, Cheong JC, Kim MK, Lee JI, In MK (2008) Simultaneous determination of amphetamine-type stimulants and cannabinoids in fingernails by gas chromatography-mass spectrometry. Arch Pharm Res 31:805–813
24. Kim JY, Shin SH, In MK (2010) Determination of amphetamine-type stimulants, ketamine and metabolites in fingernails by gas chromatography-mass spectrometry. Forensic Sci Int 194:108–114
25. Kim JY, Cheong JC, Lee JI, Son JH, In MK (2012) Rapid and simple GC-MS method for determination of psychotropic phenylalkylamine derivatives in nails using micro-pulverized extraction. J Forensic Sci 57:228–233
26. Cingolani M, Scavella S, Mencarelli R, Mirtella D, Froldi R, Rodriguez D (2004) Simultaneous detection and quantitation of morphine, 6-acetylmorphine, and cocaine in toenails: comparison with hair analysis. J Anal Toxicol 28:128–131
27. Skopp G, Potsch L (1997) A case report on drug screening of nail clippings to detect prenatal drug exposure. Ther Drug Monit 19:386–389
28. Engelhart DA, Lavins ES, Sutheimer CA (1998) Detection of drugs of abuse in nails. J Anal Toxicol 22:314–318
29. Garside D, Ropero-Miller JD, Goldberger BA, Hamilton WF, Maples WR (1998) Identification of cocaine analytes in fingernail and toenail specimens. J Forensic Sci 43:974–979
30. Ragoucy-Sengler C, Kintz P (2005) Detection of smoked cocaine marker (anhydroecgonine methylester) in nails. J Anal Toxicol 29:765–768
31. Valente-Campos S, Yonamine M, de Moraes Moreau RL, Silva OA (2006) Validation of a method to detect cocaine and its metabolites in nails by gas chromatography-mass spectrometry. Forensic Sci Int 159:218–222
32. Mari F, Politi L, Bertol E (2008) Nails of newborns in monitoring drug exposure during pregnancy. Forensic Sci Int 179:176–180
33. Garside D (2008) Drugs-of-abuse in nails. In: Jenkins A, Caplan Y (eds) Drug testing in alternate biological specimens. Forensic science and medicine. Humana Press, New York, pp 43–65
34. Jenkins AJ, Goldberger BA (1997) Identification of unique cocaine metabolites and smoking by-products in postmortem blood and urine specimens. J Forensic Sci 42:824–827
35. Ali EA, Edwards HM, Hargreaves M, Scowen I (2008) Raman spectroscopic investigation of cocaine hydrochloride on human nail in a forensic context. Anal Bioanal Chem 390:1159–1166
36. Lemos NP, Anderson RA, Valentini R, Tagliaro F, Scott RT (2000) Analysis of morphine by RIA and HPLC in fingernail clippings obtained from heroin users. J Forensic Sci 45:407–412
37. Lemos NP, Anderson RA, Robertson JR (2000) The analysis of methadone in nail clippings from patients in a methadone-maintenance program. J Anal Toxicol 24:656–660
38. Shen M, Chen H, Xiang P (2014) Determination of opiates in human fingernail—Comparison to hair. J Chromatogr B 967:84–89
39. Jones J, Jones M, Plate C, Lewis D (2013) The detection of THCA using 2-dimensional gas chromatography-tandem mass spectrometry in human fingernail clippings: method validation and comparison with head hair. Am J Anal Chem 4:1–8
40. Lemos NP, Anderson RA, Robertson JR (1999) Nail analysis for drugs of abuse: extraction and determination of cannabis in fingernails by RIA and GC-MS. J Anal Toxicol 23:147–152
41. Jones J (2012) Liquid chromatography-tandem mass spectrometry assay to detect ethyl glucuronide in human fingernail: comparison to hair and gender differences. Am J Anal Chem 3:83–91
42. Morini L, Colucci M, Ruberto MG, Groppi A (2012) Determination of ethyl glucuronide in nails by liquid chromatography tandem mass spectrometry as a potential new biomarker for chronic alcohol abuse and binge drinking behavior. Anal Bioanal Chem 402:1865–1870
43. Morini L, Marchei E, Tarani L, Trivelli M, Rapisardi G, Elicio MR, Ramis J, Garcia-Algar O, Memo L, Pacifici R, Groppi A, Danesino P, Pichini S (2013) Testing ethylglucuronide in maternal hair and nails for the assessment of fetal exposure to alcohol: comparison with meconium testing. Ther Drug Monit 35:402–407
44. Keten A, Zeren C, Arslan MM, Daglioglu N, Karanfil R, Sen BB (2013) Determination of ethyl glucuronide in fingernails by LC/MS-MS. Rom J Leg Med 21:67–72
45. Berger L, Fendrich M, Jones J, Fuhrmann D, Plate C, Lewis D (2014) Ethyl glucuronide in hair and fingernails as a long-term alcohol biomarker. Addiction 109:425–431
46. Crunelle CL, Yegles M, van Nuijs AL, Covaci A, De Doncker M, Maudens KE, Sabbe B, Dom G, Lambert WE, Michielsen P, Neels H (2014) Hair ethyl glucuronide levels as a marker for alcohol use and abuse: a review of the current state of the art. Drug Alcohol Depend 134:1–11
47. Jenkins AJ, Engelhart DA (2006) Phencyclidine detection in nails. J Anal Toxicol 30:643–644
48. Jenkins AJ, Gubanich K (2002) Disposition of citalopram in biological specimens from postmortem cases. J Forensic Sci 47:159–164
49. Al-Delaimy WK, Mahoney GN, Speizer FE, Willett WC (2002) Toenail nicotine levels as a biomarker of tobacco smoke exposure. Cancer Epidem Biomar 11:1400–1404
50. Stepanov I, Feuer R, Jensen J, Hatsukami D, Hecht SS (2006) Mass spectrometric quantitation of nicotine, cotinine, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in human toenails. Cancer Epidem Biomar 15:2378–2383
51. Stepanov I, Hecht SS, Lindgren B, Jacob P, Wilson M, Benowitz NL (2007) Relationship of human toenail nicotine, cotinine, and

4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol to levels of these biomarkers in plasma and urine. Cancer Epidem Biomar 16:1382–1386
52. Al-Delaimy WK, Willett WC (2008) Measurement of tobacco smoke exposure: comparison of toenail nicotine biomarkers and self-reports. Cancer Epidem Biomar 17:1255–1261
53. Al-Delaimy WK, Stampfer MJ, Manson JE, Willett WC (2008) Toenail nicotine levels as predictors of coronary heart disease among women. Am J Epidemiol 167:1342–1348
54. Schutte-Borkovec K, Heppel CW, Heling AK, Richter E (2009) Analysis of myosmine, cotinine and nicotine in human toenail, plasma and saliva. Biomarkers 14:278–284
55. Al-Delaimy WK, Willett WC (2011) Toenail nicotine level as a novel biomarker for lung cancer risk. Am J Epidemiol 173:822–828
56. Hsieh SJ, Ware LB, Eisner MD, Yu L, Jacob P 3rd, Havel C, Goniewicz ML, Matthay MA, Benowitz NL, Calfee CS (2011) Biomarkers increase detection of active smoking and secondhand smoke exposure in critically ill patients. Crit Care Med 39:40–45
57. Stepanov I, Hecht SS (2008) Detection and quantitation of *N*′-nitrosonornicotine in human toenails by liquid chromatography-electrospray ionization-tandem mass spectrometry. Cancer Epidem Biomar 17:945–948
58. Stepanov I, Knezevich A, Zhang L, Watson CH, Hatsukami DK, Hecht SS (2012) Carcinogenic tobacco-specific *N*-nitrosamines in US cigarettes: three decades of remarkable neglect by the tobacco industry. Tob Control 21:44–48
59. Irving RC, Dickson SJ (2007) The detection of sedatives in hair and nail samples using tandem LC-MS-MS. Forensic Sci Int 166:58–67
60. Chen H, Xiang P, Sun QR, Shen M (2012) Comparison of clozapine in nail and hair of psychiatric patients determined with LC-MS/MS. Yao Xue Xue Bao 47:1193–1199
61. Chen H, Xiang P, Shen M (2014) Determination of clozapine in hair and nail: the role of keratinous biological materials in the identification of a bloated cadaver case. J Forensic Leg Med 22:62–67
62. Choi MH, Yoo YS, Chung BC (2001) Measurement of testosterone and pregnenolone in nails using gas chromatography–mass spectrometry. J Chromatogr B 754:495–501
63. Brown HG, Perrett D (2011) Detection of doping in sport: detecting anabolic-androgenic steroids in human fingernail clippings. Med Leg J 79:67–69
64. Krumbiegel F, Hastedt M, Tsokos M (2014) Nails are a potential alternative matrix to hair for drug analysis in general unknown screenings by liquid-chromatography quadrupole time-of-flight mass spectrometry. Forensic Sci Med Pathol 10:496–503
65. Koren G, Hutson J, Gareri J (2008) Novel methods for the detection of drug and alcohol exposure during pregnancy: implications for maternal and child health. Clin Pharmacol Ther 83:631–634
66. Bandstra ES, Morrow CE, Mansoor E, Accornero VH (2010) Prenatal drug exposure: infant and toddler outcomes. J Addict Dis 29:245–258
67. Zook EG, Van Beek AL, Russell RC, Beatty ME (1980) Anatomy and physiology of the perionychium: a review of the literature and anatomic study. J Hand Surg Am 5:528–536
68. Gray T, Huestis M (2007) Bioanalytical procedures for monitoring in utero drug exposure. Anal Bioanal Chem 388:1455–1465
69. European Monitoring Centre for Drugs and Drug Addiction (2008) Sexual assaults facilitated by drugs or alcohol. http://www.emcdda.europa.eu/publications/technical-datasheets/dfsa. Accessed September 2014
70. Le Gros Clark WE, Buxton LHD (1938) Studies in nail growth. Br J Dermatol 50:221–235
71. Gilchrist ML (1939) The relation of finger-nail growth to nutritional status. J Anat 73:575–582
72. Jones FW (1942) The principles of anatomy as seen in the hand, 2nd edn. Baillaire Tindall Cox, London
73. Sibinga MS (1959) Observations on growth of fingernails in health and disease. Pediatrics 24:225–233
74. Orentreich N, Markofsky J, Vogelman JH (1979) The effect of aging on the rate of linear nail growth. J Invest Dermatol 73:126–130
75. Geyer AS, Onumah N, Uyttendaele H, Scher RK (2004) Modulation of linear nail growth to treat diseases of the nail. J Am Acad Dermatol 50:229–234
76. Yaemsiri S, Hou N, Slining MM, He K (2010) Growth rate of human fingernails and toenails in healthy American young adults. J Eur Acad Dermatol Venereol 24:420–423
77. Ropero-Miller JD, Huestis MA, Stout PR (2012) Cocaine analytes in human hair: evaluation of concentration ratios in different cocaine sources, drug-user populations and surface-contaminated specimens. J Anal Toxicol 36:390–398
78. Green SJ, Wilson JF (1996) The effect of hair color on the incorporation of methadone into hair in the rat. J Anal Toxicol 20:121–123
79. Reid RW, O'Connor FL, Deakin AG, Ivery DM, Crayton JW (1996) Cocaine and metabolites in human graying hair: pigmentary relationship. J Toxicol Clin Toxicol 34:685–690