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REVIEW ARTICLE

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Current status of keratinized matrices in Toxicology: Comparison of hair and nails

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Abstract

Nails are a keratinized matrix that has been proposed as an alternative to hair to evaluate long-term and retrospective consumption of drugs of abuse and pharmaceuticals. This matrix has been gaining interest in recent years, with new studies focusing on the analysis of fingernails and/or toenails for different substances. However, nails and hair present differences in structure, growth, and incorporation pathways that may affect drug incorporation and analysis and complicate the interpretation of the results. To better understand the results in nail samples, a comparison of concentrations found in hair, fingernails, and toenails has been described in the literature for some drugs. This review unifies the results found in the literature, with special interest on studies that report paired samples from the same individuals. Differences between fingernail and toenail samples, as well as proposed cut-offs in nails, are also discussed. Definite conclusions can be reached for some drugs, but, in general, more standardized studies are needed to better understand nail results.

KEYWORDS
alternative matrix, hair, nails, review, toxicology

1 | INTRODUCTION

Hair and nails are keratinized structures that can be used as biological matrices for the detection of endogenous and exogenous substances. Unlike conventional matrices like blood or urine, the hair and nails can incorporate and accumulate substances over a long period of time (weeks to months), offering a wide window of detection that theoretically makes them useful for many applications in the context of Clinical and Forensic Toxicology.[1] Hair analysis has been routinely applied in Toxicology for decades, for the chronological study of drug consumption, for driving license regranting,[2] in drug facilitated crimes,[3] to determine adherence to treatment,[4] or to detect drug consumption during pregnancy.[5] Nails, however, have only been studied in a handful of occasions in the past, although in the last years there has been a growing interest in this matrix. Despite both being keratinized matrices, hair and nails present some structural differences that can affect the results of the analysis and their interpretation.

1.1 | Hair and nail structure

Hair is originated in the hair follicles, where rapidly proliferating matrix cells (keratinocytes) in the hair bulb produce the hair shaft. The matrix cells differentiate through a process of keratinization, the formation of disulfide bonds and cellular dehydration, move upwards, and are compressed into their final shape.

The hair shaft is composed of three concentric layers of cells, the medulla, cortex, and cuticle. The medulla is the innermost layer and determines the final diameter of the hair; the cells are keratinized but loosely bound, with air in between. The cortex is bound to the medulla and constitutes the bulk of the hair shaft. It is composed of

long keratinized cells bound into long fibers by the tegument. Between the cells in the cortex, there are very small air spaces called *fusi*. Interspersed among the matrix cells are the melanocytes, which produce the pigment melanin. The outermost layer is the cuticle. It covers the other two and is strongly bound to the cortex. It consists of five to seven layers of long, overlapping cells that anchor the hair to the follicle and protect the internal fibers. However, the cuticle can be penetrated by aqueous solutions and be damaged by chemical products, heat, light, or mechanical injury.

Nails are originated in the nail matrix and attached to the nail bed. The nail plate is formed as matrix cells become larger and paler, and eventually the nucleus disintegrates. Melanocytes are also present in the nail matrix[6] in two compartments, the first has quiescent melanocytes unable to synthesize melanin under normal conditions, and the second is of functionally differentiated melanocytes. Nevertheless, their density is low (217 mm^2) compared with that of the epidermis.[7] In Caucasian nails, the matrix melanocytes contain premelanosomes and melanosomes I and II with little or no active synthesis of mature melanosomes.[6] The melanocytes in Japanese people contain all gradation of maturing melanosomes, and in Black subjects, most of the melanosomes are mature.[7] When the normally dormant matrix melanocytes are activated, melanin is deposited in the growing nail plate resulting in a pigmented band.[8] This is known as longitudinal melanonychia. Longitudinal melanonychia is a benign phenomenon found particularly in Afro-Caribbeans[9,10] and Japanese populations,[11] while in Caucasians, it has a higher chance of being malignant.[12]

The nail plate gains thickness and density as it grows distally. The length of the matrix seems to influence the thickness of the nail plate, along with other factors such as the linear rate of nail growth,[13] vascular supply, subungual hyperkeratosis, and the use of drugs.

Three regions of the nail plate have been defined. The dorsal plate is physically hard and has little acid phosphatase activity. It has a high calcium, phospholipid, and sulfhydryl group content. The intermediate nail plate has a high acid phosphatase activity, a high number of disulfide bonds, and a low content of bound sulfhydryl groups, phospholipid, and calcium. The existence of a ventral plate is more controversial. Jarrett and Spearman[14] define the ventral plate as a layer one or two cells thick, with the same high content in calcium, phospholipid, and sulfhydryl groups as the dorsal plate, and high acid phosphatase activity and disulfide bonds as the intermediate plate, while Jemec and Serup[15] suggest the nail plate being a bilamellar structure without a ventral layer. The nail can be compared in some aspects to a hair follicle, sectioned longitudinally, and laid on its side. The hair bulb is considered analogous to the intermediate nail matrix and the cortex to the nail plate.

1.2 | Hair and nail growth

Hair growth is not continuous but asynchronous; that is, it grows in cycles. Moreover, human hair grows in a mosaic pattern; that is, each individual follicle follows a growth cycle independently of the surrounding hair follicles.[16] The growth cycle of a hair follicle consists of four phases: a growing phase (anagen), a regression phase (catagen), a resting phase (telogen), and a shedding phase (exogen).[17] Hair grows at a different rate depending on the body site. In head hair, a range of 0.6 to 3.36 cm/month has been observed, although a mean of 1 cm/month is accepted at the posterior vertex zone (Figure S1).

Unlike hair, nails grow continuously and in two directions: 80% of the growth is longitudinal, from the nail matrix, and 20% is growth in thickness from the nail bed upwards. There are no significant growth rate differences between right and left fingernail/toenails,[18,19] although growth rates vary from finger to finger, with longer fingers showing a faster rate. In general, an average fingernail growth rate of 3 mm/month is accepted, about twice that of toenails (1.5 mm/month).[20] Thickening is constant but slow, with a mean value of 0.027 mm/month[20] (Figure S2).

1.3 | Drug incorporation into hair and nails

Substances are incorporated into the hair following a multicompartment model. The principal mechanism of incorporation is through passive diffusion of the substances from the blood irrigating the dermal papilla into the cells of the hair follicle, remaining trapped after keratogenesis, but there is also incorporation from sweat and sebum after hair formation, and from the external environment after the hair has emerged from the skin.

Passive diffusion is influenced by two factors. One is the liposolubility of the molecules: the more lipophilic, the easier the entrance through the membranes to the inside of the cells. The other is the pKa of the molecules and the cellular pH: nonionized molecules diffuse more easily through the membrane, and because the pH in the inside of melanocytes and keratinocytes is more acidic than the plasma (pH = 3–6 in the cells versus pH = 7.3 in the plasma), basic molecules can enter the cells more easily. It has been observed that drug incorporation might be augmented by binding to components in the hair cells, especially to melanin,[21,22] although this is not the only mechanism, because in albino animals there is also some incorporation of drugs to hair.[23] Another augmenting mechanism might be the binding to sulfhydryl-containing amino acids present in hair, like cysteine, especially for divalent cations that can form covalent bonds.[24] The model of passive diffusion assumes that the amount of drug incorporated depends on blood concentrations, which depend on the dose ingested. This model is also the basis for segmental analysis. Because hair is assumed to grow at a constant rate, drug disposition along the hair shaft can be correlated with the moment the drug was present in the blood flow.

On the other hand, the porous nature of the hair allows it to absorb liquids, increasing its weight and incorporating substances present in the sweat and sebum that are secreted as the hair grows. The free and nonionized drugs diffuse from blood to sweat. The pH difference between sweat (pH = 5.8) and blood (pH = 7.3) facilitates the passive diffusion of basic molecules from blood to sweat, where they are ionized, thus blocking their return to the blood.[25] Sweat and

sebum incorporation is especially relevant in the case of drugs of abuse, which are found in these secretions at high concentrations,[26] and the high interindividual variability in their secretion can explain differences in concentrations in people receiving the same dose, as well as the incorporation of drugs along the hair shaft, not corresponding to the times of administration.[26] These secretions are bound less tightly, because they occur after hair formation, so they should be easier to remove by washing the hair before the analysis. External contamination from air, water, or dust has been proposed as an incorporation path for trace elements found in hair, and it is potentially important in the case of smoked drugs such as marijuana, cocaine, and heroin.[27] If the hair is exposed to high concentrations of these contaminants, the molecules can be incorporated to the external layers of the hair. This exposition is not indicative of an active consumption of the drugs, and it should be removed by a washing procedure before the analysis to avoid false positive results.

Likewise, drugs are primarily incorporated into nails through diffusion from the blood flow, but unlike hair, nails grow in two different directions, and substances can be incorporated both from the germinal matrix and from the nail bed. Because 80% of the nail is formed in the germinal matrix, most of the incorporation occurs longitudinally, but there is also some incorporation throughout the nail bed and from sweat. Two studies about the incorporation of zolpidem in nails have found evidence of these three incorporation paths.[28,29] As with hair, incorporation into nails through external contamination, especially in fingernails due to drug handling, should be considered as a possible incorporation pathway.

The differences in the mechanism of drug incorporation in hair and nails must be taken into account for the interpretation of the results.[20] Because in the posterior vertex zone of the head hair growth is more constant, long hair samples can be divided into sections where each centimeter corresponds to 1 month of hair growth.[26] Segmentation has been studied in whole nails[30] and in one case with nail clippings,[31] but very sensitive instrumentation is needed to detect the very low concentrations present in these segments. Moreover, even if nails could be reliably divided into segments, the interpretation of the results is complex due to the double path of substance incorporation. As for structural differences, one is the presence of melanin in hair, known to influence the incorporation of certain substances depending on their physicochemical properties.[32] In Caucasians, melanin is not present in nails under normal circumstances, avoiding the bias due to pigmentation.[33] In other populations, however, the presence of melanin is more common, and its influence in the incorporation of substances has not been studied yet. The other difference is the absence of a cuticle layer in nails that can act as a barrier to substance incorporation and extraction. This means that nail samples are more susceptible to contamination from drug handling and to wash-out effects due to frequent handwashing.[34]

All these differences suggest that concentrations in hair and nail samples from the same individual could be very different, and cut-off concentrations established for hair to determine chronic consumption might not be appropriate for nails.

Nowadays, there are still few published methods for drug detection in nail samples, and even fewer publications have tackled the direct comparison of both matrices. Two reviews summarize the published methodologies for nail analysis[35] and the usefulness of nail analysis in Forensic Toxicology.[20] The present review will focus on the studies that analyze and compare drug concentrations in nail and hair samples, with special focus on paired samples, to try to elucidate the relationship between both keratinized matrices. Moreover, given the differences in growth rate and contamination pathways of fingernail and toenail samples, studies assessing the comparison between fingernails and toenails will also be reviewed.

2 | MATERIALS AND METHODS

A literature search (1980–2022) was performed using PubMed and Web of Science using combinations of the search terms nail, hair, drug, abuse, pharmaceutical, toxicology, and detection. The identified articles were reviewed and publications including the detection of substances in both nail and hair samples were selected. Only articles written in English were included.

3 | RESULTS

3.1 | Comparison of concentrations in nail and hair samples

A total of 29 publications were found that compare concentrations between nail and hair samples (Table 1). The results are separated by compound class and described in detail in the following sections.

3.1.1 | Amphetamine derivatives

Amphetamine derivatives have been studied in six publications[36–41] with varying results. In the first study, amphetamine and methamphetamine were analyzed in samples from nine suspects of drug abuse, finding similar mean concentrations in hair and nails; moreover, in paired samples, the matrix with the highest concentration was variable.[36] Another study[37] analyzed 12 paired hair and nail samples from regular users and found the same variability for these drugs. Cirimele et al.[38] analyzed hair and fingernails from one drug user and found concentrations of amphetamine, MDA and MDMA slightly higher in fingernails than in hair. Lin et al.[39] analyzed fingernails from 97 drug users, finding 62 positive cases for amphetamine and/or methamphetamine. From six of the positive subjects, they collected paired nail and hair samples and found concentrations in nail clippings lower than in the corresponding 1.5 cm hair samples for both drugs. Madry et al.[40] analyzed hair and nail samples from 15 subjects after the administration of two doses of MDMA. Concentrations for MDMA and MDA were higher in hair in most samples, and similar or lower in two and three, respectively. Finally, Cappelle et al.[41] analyzed hair, fingernails,

TABLE 1 Summary of published methods comparing concentrations in hair and nail samples.

Reference	Analyte	Samples	Paired	Comparison
Amphetamines				
Suzuki 1984[36]	AMP, MAMP	Hair ($n = 7$) Fingernail ($n = 8$) Toenail ($n = 4$)	Yes	Hair ~ nails
Suzuki 1989[37]	AMP, MAMP	Hair ($n = 15$) Nail ($n = 20$)	Yes	Hair > nails ($n = 4/9$) for MAMP Hair > nails ($n = 2/3$) for AMP
Cirimele 1995[38]	AMP, MDA, MDMA	Hair ($n = 1$) Fingernail ($n = 1$)	Yes	Fingernails > Hair
Lin 2004[39]	AMP, MAMP	Hair ($n = 6$) Fingernail ($n = 97$)	Yes	Hair > fingernails
Madry 2016[40]	MDA MDMA	Hair ($n = 13$) Fingernail ($n = 15$) Toenail ($n = 3$)	Yes	Hair (5 cm) > nail Hair ~ nail in $n = 2$
Cappelle 2018[41]	AMP, MAMP, MDMA, MDEA	Hair ($n = 26$) Fingernail ($n = 24$) Toenail ($n = 18$)	Yes	Nails > hair
Antidepressants and benzodiazepines				
Irving 2007[42]	ALP, CLOB, CLON, DIA, MID, OXA, TEM, TRIAZ, ZOP	Hair ($n = 21$) Nail ($n = 21$)	Yes	Hair ~ nails
Hang 2013[28]	ZOL	Hair ($n = 7$) Fingernail ($n = 7$)	Yes	Hair > nails
Madry 2014a[29]	ZOL	Hair ($n = 9$) Fingernail ($n = 9$)	Yes	Hair > nails
Cobo-Golpe 2021a[34]	VENL, TRAZ, CITA, PARO, FLUO, SERT, ZOL, OXA, ALP, LORA, NDIA, DIA	Hair ($n = 16$) Fingernail ($n = 16$) Toenail ($n = 17$)	Yes	Hair > nails if active treatment, except for DIA
Antipsychotics				
Uematsu 1989[43]	HALO	Hair ($n = 40$) Nail ($n = 20$)	Yes	Hair > nails
Uematsu 1990[23]	HALO	Hair ($n = 20$) Nail ($n = 20$)	Yes	Hair > nails
Chen 2012[44]	CLOZ	Hair ($n = 16$) Nail ($n = 16$)	Yes	Hair > nails
Chen 2014[45]	CLOZ	Hair, fingernails, toenails from cadaver case ($n = 1$)	Yes	Hair > nails
Cobo-Golpe 2020[46]	QUET, HALO, LMZ, CLOZ, OLAN	Hair ($n = 13$) Fingernail ($n = 13$) Toenail ($n = 13$)	Yes	Hair > nails, except for OLAN
Cannabis				
Jones 2013[47]	THCCOOH	Hair ($n = 60$) Nail ($n = 60$)	Yes	Nails > hair
Cobo-Golpe 2021b[48]	CBN, CBD, THC	Hair ($n = 17$) Fingernail ($n = 22$) Toenail ($n = 19$)	Yes	Fingernails > hair Hair > toenails
Cocaine and opioids				
Ropero-Miller 2000[49]	AEME, BE, COC, CE, EEE, EME, NBE, NCOC, 6-AM, COD, MOR, NCOD, NMOR	Hair ($n = 8$) Fingernail ($n = 8$)	Yes	Hair > nails
Cingolani 2004[50]	MOR, 6-AM, COC	Hair ($n = 18$) Toenail ($n = 18$)	Yes	Toenail > hair for COC, MOR Toenail ~ hair for 6-AM
Shen 2014[51]	6-AM, MOR, COD, AC, heroin	Hair ($n = 18$) Fingernail ($n = 18$)	Yes	Hair > nails for 6-AM, AC, COD Nail > hair for MOR

TABLE 1 (Continued)

Reference	Analyte	Samples	Paired	Comparison
Madry 2014b[33]	COC, BE, NCOC, CE	Hair ($n = 20$) Toenail ($n = 20$)	Yes	Hair > toenails
Cappelle 2018[41]	COD, MOR, 6-AM, MTD, EDDP, COC, BE, EME	Hair ($n = 26$) Fingernail ($n = 24$) Toenail ($n = 18$)	Yes	Nails > hair Hair > nails for COC
Tzatzarakis 2015[52]	BUP, NBUP	Hair ($n = 46$) Fingernail ($n = 46$)	Yes	Nails > hair for BUP Hair ~ nails for NBUP
EtG				
Jones 2012[53]	EtG	Hair ($n = 570$) Fingernail ($n = 561$)	Yes	Nails > hair
Cappelle 2017[54]	EtG	Hair ($n = 45$) Fingernail ($n = 41$) Toenail ($n = 13$)	Yes	Nails > hair
Fosen 2017[55]	EtG	Hair($n = 40$) Fingernail ($n = 40$)	Yes	Nails > hair
Paul 2019[56]	EtG	Hair ($n = 50$) Fingernail ($n = 50$)	Yes	Nails > hair in paired positive samples Hair ~ nails considering all samples
Other				
Faergemann 1993[57]	Terbinafine	Hair ($n = 12$) Nail ($n = 12$)	Yes	Hair > nails
Kim 2020[58]	COT, 3-HCOT	Hair ($n = 26$) Nail ($n = 26$)	Yes	Hair > nails
Krumbiegel 2016[30]	76 drugs	Hair ($n = 7$) Nail ($n = 7$) Postmortem cases	Yes	Concentrations not comparable Segmental analysis of nails showed different drug concentration per segment

Abbreviations: AC, acetyl codeine; AEME, anhydroecgonine methyl ester; ALP, alprazolam; AMP, amphetamine; BE, benzoylecgonine; BUP, buprenorphine; CBD, cannabidiol; CBN, cannabinol; CE, cocaethylene; CITA, citalopram; CLOB, clobazam; CLON, clonazepam; CLOZ, clozapine; Cmax, maximum concentration; COC, cocaine; COT, cotinine; DIA, diazepam; EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; EEE, ecgonine ethyl ester; EME, ecgonine methyl ester; EtG, ethylglucuronide; HALO, haloperidol; LMZ, levomepromazine; LORA, lorazepam; MDA, 3,4-methylendioxiamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine; MDMA, 3,4-methylendioximethamphetamine; MID, midazolam; MOR, morphine; MTD, methadone; NAMP, noramphetamine; NBE, norbenzoylecgonine; NBUP, norbuprenorphine; NCOC, norcocaine; NCOD, norcodeine; NDIA, nordiazepam; NMOR, normorphine; OLAN, olanzapine; OXA, oxazepam; QUET, quetiapine; TEM, temazepam; THCCOOH, tetrahydrocannabinolic acid; THC, tetrahydrocannabinol; TRIAZ, triazolam; ZOL, zolpidem; ZOP, zopiclone; 3-HCOT, 3-hydroxycotinine; 6-AM, 6-acetylmorphine.

and toenails from 26 patients in treatment for drug abuse and found higher concentrations of amphetamine, MDMA, and MDEA in nails than in hair.

3.1.2 | Cannabinoids

Two publications detected cannabinoids in paired hair and nail samples.[47,48] Cobo-Golpe et al.[48] analyzed paired fingernail, toenail, and hair samples from 23 chronic cannabis users and found THC, CBN, and CBD concentrations 4.9 to 21.2 times higher in fingernails than in hair. Conversely, concentrations in toenails were lower than in hair. Jones et al.[47] analyzed paired samples from 60 subjects and found THCCOOH concentrations in fingernails 4.3 times higher than in hair. Because THCCOOH is an endogenous metabolite of THC, the higher concentrations in fingernails cannot be attributed to external contamination but rather to a greater accumulation in nails over time, considering the slower growth of nails compared with hair.

3.1.3 | Cocaine

For cocaine, four publications compare concentrations in hair and nails.[33,41,49,50] Three of them found higher concentrations in hair than nails,[31,41,49] and one[50] found higher concentrations in nails. Ropero-Miller et al.[49] analyzed hair and fingernail scrapings from eight participants in an in-patient study. Participants received alternating low doses of cocaine and codeine for 6 days, placebo for 2 weeks and a high dose for another week. The analytes were detected in the nail washing solution, but not in the washed nail scrapings. Considering the total drug detected (sum of sample concentration and washes concentration), the maximum concentrations were five to 30 times higher in hair than nails. Madry et al.[33] analyzed paired hair (1.5–6 cm) and toenail samples from cocaine users. Cocaine concentrations in hair were two to 42 times higher than toenail clipping concentrations (median = 16 times); however, the sum of metabolites (benzoylecgonine [BE], norcocaine, and cocaethylene) hair concentrations were only 0.6 to nine times higher than toenails (median = 2.5 times).

Higher concentrations in hair were attributed to melanin binding and external contamination in the case of the parent compound. Cappelle et al.[41] found higher concentrations in hair for cocaine, but concentrations for its metabolites BE and ecgonine methyl ester (EME) were higher in nails ($n = 26$). Cingolani et al.[50] detected cocaine in hair and toenail samples from 18 autopsies of drug abusers. Entire nails were removed from the big toe, and the proximal portion of both nails and hair was used for the analysis. Mean concentrations of cocaine in toenails were twice those in hair.

3.1.4 | Cotinine

Kim et al.[58] analyzed hair and nail samples from 26 infants to determine second-hand tobacco exposure. They detected the nicotine metabolites cotinine and hydroxycotinine in eight and two paired samples, respectively. Concentrations of both analytes were higher in hair than in nails.

3.1.5 | Ethylglucuronide

Ethylglucuronide (EtG) concentrations were compared in four publications.[53–56] All of them found higher concentrations in nails (1.5 to five times higher, depending on the study). Jones et al.[53] analyzed paired hair and fingernails of 529 participants. EtG concentrations were higher in fingernails, with mean concentration three times higher than in hair. Cappelle et al.[54] analyzed samples from 45 patients in treatment for alcohol dependency. Of these, 41 had paired fingernail-hair samples and 13 had paired toenail-hair samples. Median concentrations in nail samples were higher than in hair (1.5 and 4.2 times higher in fingernails and toenails, respectively). Fosen et al.[55] analyzed paired hair and nails from 40 patients of an alcohol rehabilitation clinic. Median concentrations of EtG were five times higher in nails than hair. Finally, Paul et al.[56] analyzed paired hair and fingernails from 50 participants. The mean EtG concentrations in hair and fingernails were similar when considering all cases, but for those positive in both matrices ($n = 21$), EtG concentrations were 1.5 times higher in nail samples.

3.1.6 | Opioids

There are five publications comparing opioid compounds in both matrices.[41,49–52] Morphine was detected in three of them, all at higher concentrations in nails[41,50,51]; codeine was detected in higher concentrations in hair in two publications,[49,51] and lower in another[41]; and 6-acetylmorphine (6-AM) was detected in similar concentrations in both matrices in one study,[50] higher in hair in another,[51] and lower in the last one.[41] Specifically, Shen et al.[51] analyzed fingernail and hair samples from 18 subjects whose urine had tested positive for morphine. Morphine concentrations were higher in nails, while 6-AM, codeine, and acetylcodeine were higher in hair. Cingolani et al.[50] found mean concentrations of morphine 1.6 times higher in toenails than hair, but mean concentrations of 6-AM were similar in both matrices. Cappelle et al.[41] detected morphine, 6-AM, codeine, methadone, and its metabolite EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine) and found higher concentrations in nails (fingernails and toenails) than hair. Buprenorphine was studied only by Tzatzarakis et al.,[52] who analyzed samples from 46 patients and found higher median concentrations of buprenorphine in fingernails than hair, but similar concentrations in both matrices for its metabolite norbuprenorphine.

3.1.7 | Antidepressants and benzodiazepines

There are five publications comparing antidepressants or benzodiazepines in nails and hair. Two of them focused on the detection of zolpidem after the administration of a single dose.[28,29] Madry et al.[29] investigated concentrations in paired hair and fingernail samples from nine volunteers. Comparing concentrations in the segments corresponding to incorporation at the moment of administration, they found concentrations two to 10 times higher in hair than nails. Hang et al.[28] studied zolpidem concentrations in paired fingernails and hair from seven subjects and found that hair concentrations were around 1000 times higher. The other three publications included multiple drugs. Krumbiegel et al.[30] investigated 76 substances in hair and nails from seven postmortem cases. The nail and hair samples were segmented differently in each case, making it difficult to compare concentrations in the two matrices. The analytes of interest detected in these cases were zolpidem, diazepam, nordiazepam, and oxazepam; the first three were found in higher concentrations in hair, whereas for oxazepam, results varied. Other drugs of abuse and pharmaceuticals were detected in the samples, with high variability in the results between sample segments and individual cases. Irving and Dickson[42] detected alprazolam, clobazam, clonazepam, diazepam, midazolam, oxazepam, temazepam, triazolam, zopiclone, and some metabolites in hair, fingernails, and toenails of 21 patients that had been prescribed any of the drugs. In general, concentrations were similar in hair and nail samples, but for each drug, a pattern could usually be observed. For example, for N-desmethyl clobazam, diazepam, nordiazepam, and triazolam, concentrations were higher in nails, while for midazolam, temazepam, and zopiclone, concentrations were higher in hair. Cobo-Golpe et al. described hair and nail concentrations for 12 drugs, namely, venlafaxine, trazodone, citalopram, paroxetine, fluoxetine, sertraline, zolpidem, oxazepam, alprazolam, lorazepam, nordiazepam, and diazepam,[34] although comparison between the two matrices was only possible for some of them. Hair concentrations were higher than nail concentrations in patients under active treatment with trazodone, sertraline, alprazolam, zolpidem, venlafaxine, paroxetine, and fluoxetine. Diazepam was the only exception, with higher concentrations in nails in the only case available.

3.1.8 | Antipsychotics

Five publications show antipsychotic drugs concentrations in nail and hair samples. Uematsu et al.[23,43] found much higher concentrations of

TABLE 2 Summary of published methods comparing concentrations in fingernail and toenail samples.

Reference	Analyte	Samples	Paired	Comparison
Amphetamines				
Suzuki 1984[36]	AMP, MAMP	Fingernail ($n = 8$) Toenail ($n = 4$)	Yes	Toenails > fingernails
Madry 2016[40]	MDA MDMA	Fingernail ($n = 15$) Toenail ($n = 3$)	Yes	Fingernails ~ toenails
Cappelle 2018[41]	AMP, MAMP, MDMA, MDEA	Fingernail ($n = 24$) Toenail ($n = 18$)	Yes	Toenails > fingernails for AMP, MDMA
Shu 2015[59]	AMP, MAMP	>10,000 cases	No	Fingernails ~ toenails (Median concentrations)
Antidepressants and benzodiazepines				
Hang 2013[28]	ZOL	Fingernail ($n = 7$) Toenail ($n = 4$)	Yes	Toenails > fingernails
Cobo-Golpe 2021a[34]	VENL, TRAZ, CITA, PARO, FLUO, SERT, ZOL, OXA, ALP, LORA, NDIA, DIA	Fingernail ($n = 16$) Toenail ($n = 17$)	Yes	Toenails ~ fingernails for ZOL, VENL Toenails > or ~ fingernails for ALP, TRAZ Toenails > fingernails for FLUO, SERT
Shu 2015[59]	ALP, DIA, NDIA	>10,000 cases	No	Fingernails ~ toenails (Median concentrations)
Antipsychotics				
Chen 2014[45]	CLOZ	Fingernails, toenails from cadaver case ($n = 1$)	Yes	Fingernails > toenails
Cobo-Golpe 2020[46]	QUET, HALO, LMZ, CLOZ, OLAN	Fingernail ($n = 13$) Toenail ($n = 12$)	Yes	Toenails > or ~ fingernails depending on the participant
Cannabis				
Cobo-Golpe 2021b[48]	CBD, CBN, THC	Fingernail ($n = 22$) Toenail ($n = 19$)	Yes	Fingernails > toenails (Median concentrations)
Shu 2015[59]	THCCOOH	>10,000 cases	No	Fingernails ~ toenails (Median concentrations)
Cocaine and opioids				
Garside 1998[60]	COC, BE	Fingernail ($n = 14$) Toenail ($n = 14$) Postmortem cases	Yes	Fingernails > toenails for COC Fingernails > or ~ toenails for BE
Engelhart 2002[61]	COC, BE, EME, NCOC, CE, MOR, 6-AM, COD	Fingernail ($n = 17$) Toenail ($n = 17$) Postmortem cases	Yes	Fingernails > toenails Fingernails ~ toenails for COD
Cappelle 2018[41]	COC, BE, EME, COD, MOR, 6-AM, MTD, EDDP	Fingernail ($n = 24$) Toenail ($n = 18$)	Yes	Fingernails > toenails Toenails > fingernails for COD, MTD
Shu 2015[59]	COC, BE, NCOC, CE, 6-AM, MOR, COD, HCOD, HYM, MTD, EDDP, OXC, OXM	>10,000 cases	No	Fingernails > toenails
EtG				
Cappelle 2017[54]	EtG	Fingernail ($n = 41$) Toenail ($n = 13$)	Yes	Toenails > fingernails (Mean concentrations)
Shu 2015[59]	EtG	>10,000 cases	No	Fingernails > toenails (Mean and median concentrations)
Other				
Jenkins 2006[62]	PCP	Fingernail ($n = 4$) Toenail ($n = 4$) Postmortem cases	Yes	Fingernails > toenails

Abbreviations: ALP, alprazolam; AMP, amphetamine; BE, benzoylecgonine; CBD, cannabidiol; CBN, cannabinol; CE, cocaethylene; CITA, citalopram; CLOZ, clozapine; Cmax, maximum concentration; COD, codeine; COC, cocaine; DIA, diazepam; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; EME, ecgonine methyl ester; EtG, ethylglucuronide; HALO, haloperidol; HCOD, hydrocodone; HYM, hydromorphone; LMZ, levomepromazine; LORA, lorazepam; MDA, 3,4-methylendioxiamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine; MDMA, 3,4-methylendioximethamphetamine; MOR, morphine; MTD, methadone; NCOC, norcocaine; NDIA, nordiazepam; OLAN, olanzapine; OXA, oxazepam; OXC, oxycodone; OXM, oxymorphone; PCP, phencyclidine; QUET, quetiapine; THCCOOH, tetrahydrocannabinolic acid; THC, tetrahydrocannabinol; ZOL, zolpidem; 6-AM, 6-acetylmorphine.

haloperidol in hair than nails (concentrations in nails about 3%–4% those in hair) in two different studies when comparing paired samples from 20 patients after receiving a fixed daily dose for more than a month. Chen et al. found nail concentrations about 5%–10% those in hair, after analyzing hair and fingernail samples from 16 psychiatric patients[44] and in one case of a drowned cadaver.[45] More recently, Cobo-Golpe et al.[46] published an article detecting five antipsychotic drugs (quetiapine, haloperidol, levomepromazine, clozapine, and olanzapine) in hair and nail samples from psychiatric patients under chronic treatment. Hair concentrations of quetiapine, haloperidol, levomepromazine, and clozapine were higher than nail concentrations, whereas results for olanzapine were very variable. In general concentrations of antipsychotics were higher in hair, most likely due to melanin binding in hair samples.

3.1.9 | Terbinafine

Faergemann et al.[57] measured terbinafine concentrations in paired hair and toenails from 12 participants during and after ingesting a daily dose for 28 days. Mean concentrations of terbinafine were 2.3 to 9.6 times higher in hair than nails during the study.

3.2 | Comparison of concentrations in fingernail and toenail samples

Fingernail and toenail samples differ in growth rates and possible contamination pathways. This could lead to different concentrations in both samples and should be taken into account when choosing the type of nail sample to analyze. Thirteen publications describe the comparison between fingernail and toenail concentrations (Table 2), and the results are detailed in the following sections.

3.2.1 | Amphetamine derivatives

Amphetamine derivatives were compared in fingernail and toenail in four studies,[36,40,41,59] finding discrepant results. Madry et al.[40] found similar MDA and MDMA concentrations in three toenail samples compared with those in 13 fingernail samples, with one outlier that was attributed to a past consumption of MDMA more than 2 months before the study. Shu et al.[59] analyzed nail samples from more than 10,000 high-risk cases, detecting 52 different drugs of abuse. The median concentrations for amphetamines were also similar in both nail samples. However, Suzuki et al.[36] found higher concentrations for amphetamine and methamphetamine in toenails than fingernails in nine subjects. Similarly, Cappelle et al.[41] found concentrations of amphetamine and MDMA higher in toenails than fingernails in 26 patients in treatment for drug abuse.

3.2.2 | Cannabinoids

Only two studies compared concentrations in fingernail and toenail samples. Cobo-Golpe et al.[48] analyzed paired fingernail and toenail samples from 23 chronic cannabis consumers and found much higher concentrations in fingernails than in toenails (eight to 29 times higher) for THC, CBD, and CBN. Shu et al.[59] found similar THCCOOH concentrations in fingernails and in toenails, although due to the high number of samples, the authors did not compare paired samples but the range and median of concentrations.

3.2.3 | Cocaine and opioids

Cocaine[59–61] and opioids[41,59,61] concentrations in nail samples were compared in three studies each.

For cocaine and opioids, fingernails tend to have higher concentrations than toenails, but with some exceptions. Specifically, Garside et al.[60] analyzed paired fingernail and toenail samples from 14 postmortem cases of suspected cocaine users. Cocaine and BE were the analytes predominantly detected; cocaine concentrations were higher in fingernails than toenails, with some samples being positive in fingernails while negative in toenails. BE was also higher in fingernails in some cases, but similar concentrations were found in others. Engelhart and Jenkins[61] analyzed cocaine analytes and opiates in paired fingernails and toenails from 17 postmortem cases. For cocaine and its metabolites (BE, EME, norcocaine, cocaethylene), concentrations in fingernails were seven to 20 times higher than in toenails. The opioids majorly detected were morphine, 6-AM, and codeine; concentrations of morphine and 6-AM were 30 times greater in fingernails, while codeine concentrations did not differ significantly between both matrices. Shu et al.[59] found mean and median concentrations of cocaine and opioids higher in fingernail samples. Cappelle et al.[41] found cocaine, BE, EME, morphine, 6-AM, and EDDP higher in fingernails and codeine and methadone higher in toenails ($n = 26$).

3.2.4 | EtG

EtG was only studied by Shu et al.,[59] who found mean and median concentrations higher in fingernail samples.

3.2.5 | PCP

PCP was investigated by Jenkins and Engelhart,[62] who analyzed nail samples from four postmortem cases that tested positive for PCP in urine. Paired fingernails and toenails were available in three individuals, with PCP concentrations higher in fingernails.

3.2.6 | Antidepressants and benzodiazepines

Three authors compared concentrations in both nail samples for these drugs. Hang et al.[28] studied zolpidem concentrations in paired fingernails and toenails from seven subjects after the administration of a single dose. Concentrations in toenails were higher than in fingernails (up to three times). Cobo-Golpe et al.[34] studied different antidepressant and benzodiazepine drugs in 21 patients and found similar concentrations in fingernail and toenail samples when considering patients under active treatment. Specifically, concentrations for zolpidem, venlafaxine, and two samples containing trazodone were similar in both nail samples, and in one case, trazodone concentrations were five times higher in toenails than fingernails. For alprazolam, one case showed similar concentrations in both samples, while another was positive in toenails but negative in fingernails. Fluoxetine and sertraline concentrations were higher in toenails ($n = 1$) (1.75 and 1.5 times higher, respectively). Shu et al.[59] analyzed nail samples from more than 10,000 high-risk cases, detecting 52 different drugs of abuse. The range of concentrations for alprazolam, diazepam, and nordiazepam was similar.

3.2.7 | Antipsychotics

Fingernail and toenail concentrations for antipsychotic drugs were only compared in two studies. Cobo-Golpe et al.[46] compared samples from 13 patients and found similar or higher concentrations in toenails for all analytes except olanzapine and quetiapine, for which one sample and four samples, respectively, showed higher concentrations in fingernails. Chen et al.[45] found mean concentrations of clozapine in fingernails slightly higher (1.3 times) than in toenails from a bloated cadaver case.

3.3 | Cut-offs in nail samples

Due to the differences in concentrations between hair, fingernails, and toenails, the cut-off concentrations established for hair are not valid to interpret the concentrations found in nails. To date, only five studies have proposed preliminary cut-off concentrations in nail samples for EtG, THC, amphetamine, cocaine, BE, and EME (Table 3).

Regarding EtG, Berger et al.[63] analyzed 547 hair samples and 506 fingernail samples, paired when both samples were available. Concentrations of EtG were always higher in fingernails than in hair, and the cut-off for excessive alcohol consumption proposed in fingernails was 56 pg/mg. Cappelle et al.[54] analyzed 45 hair, 41 fingernail, and 13 toenail samples (paired when possible) from patients treated for alcohol use disorders. Higher concentrations were present in fingernails and toenails compared with hair. The study proposes preliminary cut-off values for EtG concentrations in fingernails: >123 pg/mg for chronic excessive alcohol consumption, 59–123 pg/mg for moderate alcohol consumption, and <59 pg/mg for alcohol abstinence. Fosen et al.[55] also detected higher EtG concentrations in fingernails than in hair and proposed that the cut-off in fingernails should be higher than in hair, but considered that the number of samples ($n = 40$) was not enough to calculate a cut-off. More recently, a study by Vermeulen et al.[64] analyzed hair and fingernails from 111 teetotalers and proposed a cut-off for alcohol abstinence based on the measured EtG concentrations in fingernails in their study. EtG concentrations were lower than the LOQ of 2.1 pg/mg in most samples, and the highest concentration was 23 pg/mg. The authors proposed the 97.5% percentile of 7.6 pg/mg as a cut-off for alcohol abstinence.

Cobo-Golpe et al.[46] analyzed 22 fingernail, 19 toenail, and 16 hair samples from chronic cannabis users, finding a better correlation of hair concentrations with toenails, and proposed a THC cut-off in toenails of 16.5 pg/mg.

Finally, Cappelle et al.[41] analyzed hair and nail samples from inpatients engaged in a treatment program for substance use disorders. Twenty-six hair samples, 24 fingernail samples, and 18 toenail samples were collected. Concentrations were higher in nails for BE, EME, and amphetamine, while for cocaine, concentrations were higher in hair. Cut-off values were proposed in fingernails and toenails for cocaine (440 and 150 pg/mg), BE (175 and 105 pg/mg), EME (80 and 40 pg/mg), and amphetamine (485 and 505 pg/mg, respectively).

4 | DISCUSSION

When comparing concentrations found in hair with those in nails, some drugs showed a clear pattern. This is the case of

TABLE 3 Cut-off concentrations proposed for different drugs in nail samples.

Reference	Drug	Concentrations	Cut-off	Hair (pg/mg)	Fingernail (pg/mg)	Toenail (pg/mg)
Cappelle 2017[54]	EtG	Toenail > fingernail > hair	Excessive consumption	>30	>123	-
			Abstinence	<7	<59	-
Berger 2014[63]	EtG	Fingernail > hair	Excessive consumption	>30	>56	-
Vermeulen 2022[64]	EtG	-	Abstinence	<5	<7.6	-
Cappelle 2018[41]	COC	Hair > fingernail > toenail	Chronic use	500	440	150
	BE	Fingernail > toenail > hair	Chronic use	50	175	105
	EME	Fingernail > hair > toenail	Chronic use	50	80	40
	AMP	Toenail > fingernail > hair	Chronic use	200	485	505
Cobo-Golpe 2020[46]	THC	Fingernail > hair > toenail	Chronic use	50	-	16.5

Abbreviations: AMP, amphetamine; BE, benzoylecgonine; COC, cocaine; EME, ecgonine methyl ester; EtG, ethyl glucuronide; THC, tetrahydrocannabinol.

antidepressants, benzodiazepines, antipsychotics, terbinafine, and cotinine, which have been found in higher concentrations in hair than in nails, while EtG has always been found in higher concentrations in nails. On the other hand, for most drugs of abuse, the distribution is not so clear. Amphetamine derivatives, cocaine, and its metabolites and opioids have been reported to be at higher concentrations in hair in some studies, higher in nails in others, and even different across participants from the same study. Furthermore, in the case of cannabinoids, while THCCOOH has been reported in higher concentrations in nails, for THC, CBD, and CBN, concentrations in hair were higher than in toenails, but much lower than in fingernails.

One of the main differences between these two matrices is the lack of melanin in nails in most individuals. The different affinities of each substance for melanin binding could explain not only differences in concentrations in different hair colors but also the differences in concentrations between hair and nails.

Studies of drug incorporation into hair depending on melanin content have been performed for different analytes. Lee et al.[65] studied codeine and morphine concentrations in rat hair and found that in dark gray hair concentrations were always higher than those in white hair. Ramirez-Fernandez et al.[66] evaluated the effect of melanin in antipsychotics concentrations in human hair and found that hair melanin has a much higher affinity for these drugs than hair proteins. Other authors measured the binding of the substances to melanin in in vitro experiments. Joseph et al.[67] concluded that melanin was the primary hair constituent responsible for both nonspecific and specific binding of cocaine. Gautam et al.[68] determined that, in vitro, 32% of amphetamine bound to eumelanin.

Melanin content in hair was quantified by two authors to better search for its correlation with substance incorporation.[69,70] Scheidweiler et al.[69] found that BE concentrations correlated with melanin content, and a positive linear relationship was found between total hair melanin content and Cmax for codeine, cocaine, and metabolites following high dosing. The authors suggest that the correlation with melanin content could be better explained by the melanin role in drug incorporation of these drugs inside the hair cell than because of the direct union of melanin with these compounds. The melanin role in drug incorporation was evaluated by in vitro studies, which suggest that pigmented melanocytes contain a transport system responsible for drug influx and efflux.[71] Polettini et al.[70] also found a strong correlation between the melanin concentration in hair and the AUC and Cmax of AMP and MAMP. Conversely, the incorporation of neutral and acidic compounds in hair did not appear to be melanin-correlated, as observed for THC and THCCOOH,[21,72] N-acetylamphetamine,[73] phenobarbital,[74] carbamazepine,[75] ethyl glucuronide,[76] and fatty acid ethyl esters.[77]

From the results available on nail and hair concentrations, we can infer that the affinity of each analyte for melanin plays an important role when comparing both samples. Taking this into account, basic substances, such as cocaine, codeine, antipsychotics, terbinafine, and cotinine, which have a high affinity for melanin, are found at higher concentrations in hair. Conversely, compounds with no relevant affinity for melanin, such as cannabinoids and EtG, are often detected at higher concentrations in nails due to the slower growth of nails that allows for the accumulation of the substances over time. An exception to this is seen for morphine, 6-AM, and amphetamines. These are basic substances that have been observed to accumulate in higher concentrations in darker hair due to melanin binding.[73,78] However, when comparing nail and hair concentrations, their distribution in both matrices is equivocal; morphine has been detected in higher concentrations in nails in all studies,[41,49–51] and for 6-AM[41,49–51] and amphetamines,[36–41] the matrix with higher concentrations fluctuates between the different cases and studies.

Another important difference between hair and nails to consider is the growth rate: While in head hair 1 cm/month is accepted, nails grow slower (about 3 mm/month the fingernails and 1.5 mm/month the toenails) and, therefore, substances can be incorporated over longer periods of time.[20] This means that hair, fingernail, and toenail samples collected at the same time represent different detection windows. Furthermore, nail growth is constant, but in two directions, which means that substance incorporation is different than in hair, as previously described.[28,29] Hang et al.[28] found that zolpidem appeared in the overhang of the nails just 1 week after a single dose was administrated, and the concentrations decreased over the next 6–12 weeks until a peak of concentration appeared between the 10th–15th weeks. Zolpidem stopped being detected at the 18th–30th weeks. Madry et al.[29] found the highest peak concentration after 24 h, another increase of concentrations after 2–3 weeks, and a final concentration peak after 10–18 weeks. A possible explanation for the first peak concentration is the incorporation into nails from sweat shortly after taking the dose of zolpidem. However, because the water content of nails is 9%–10%, diffusion of zolpidem through the nail bed to the free edge could also be considered.[79] After 2 weeks, a second peak is detected. At this point in time, the part of the nail that was in contact with the nail bed during zolpidem intake should be at the free edge, and the wash out effect should have eliminated the zolpidem incorporated through sweat. After peak two, there is a decrease in concentrations attributed to wash-out by daily hygiene. Finally, 10 to 18 weeks after the intake, a third peak of concentrations can be detected, corresponding to the drug incorporated from the nail matrix into the forming nails, in line with the average nail growth. In single nails, zolpidem was detected until the 20th week. This difference in incorporation pathways can also obscure the detection window of the nails. In fact, in most studies, paired hair and nail samples were collected at the same time, with no detailed information about patterns of consumption, which hinders direct comparison of concentrations. A possible solution is performing controlled studies, where one or more doses are administered, and samples are collected over a period of time. Hang et al.[28] compared zolpidem concentrations at the proximal 2 cm of hair with the peak concentrations observed in nails (10–15 weeks) and found higher concentrations in hair. Madry et al.[29] also compared zolpidem peak concentrations found in hair and nails and reported a big interindividual variability also with higher concentrations in hair, although when comparing total concentrations (sum of concentrations of each hair sample and nail sample), they did not find a difference between hair and nails. Ropero-Miller et al.[49]

administered multiple doses of cocaine and codeine and collected multiple hair and nail samples, making it possible to determine a time when the maximum concentration was observed (3–4 weeks for hair and 1–3 weeks for nails) and to compare maximum concentrations. In another study, Madry et al.[40] administered two doses of MDMA to 15 subjects and collected samples a median of 20 days after the last administration. The concentrations compared corresponded to the last 5 months in hair and 3.5 months in nails. This included the time of administration, but some amount of drug from before the start of the study could be influencing the results. Furthermore, these studies[28,29,40] show that incorporation through the nail bed makes some amount of the drug appear in nail clippings before the peak of maximum concentration, making temporal interpretation of concentrations in nails, and the comparison with hair, very difficult.

Finally, the absence of cuticle in nails can also contribute to a difference in concentrations. Incorporation through sweat can be more relevant in toenail samples, and contamination through contact during drug manipulation can affect fingernail concentrations.[48] On the other hand, the lack of a protective layer can also allow for drug extraction from the inside of the nails during normal hygiene[40] or in the decontamination step during the analysis.[49]

The choice of fingernails or toenails for the analysis must also be considered when interpreting the results. In general, concentrations in fingernails and toenails tend to be similar, although in most cases (amphetamines, antidepressants, benzodiazepines, antipsychotics), concentrations in toenails are slightly higher. This can be explained by the differences detailed for the comparison of hair and nails: an accumulation due to a slower growth rate, a higher incorporation through sweat, and a lower extraction compared with the frequent handwashing.

However, other drugs such as cannabinoids, PCP, cocaine, and opioids were detected in higher concentrations in fingernails in almost all studies. For THC, CBD, and CBN, the proposed explanation was that fingernails could have been contaminated during drug manipulation, and the washing procedure was not enough to mitigate this contamination.[48]

In the end, it is difficult to compare the different studies because of the multitude of variables that can affect concentrations in hair and nails. One of them is the dosage, as some authors study single dose consumption,[28,29] others study concentrations in chronic users (with different patterns of consumption, that are usually unknown[30,36–39,47,51,53] or estimated retrospectively[33,41,48,54–56]) and, in the case of pharmaceutical drugs, the prescribed doses also varied in time and between participants.[34,46] The election of fingernails or toenails as the nail matrix for comparison is also an important variable that is sometimes not correctly specified or considered when interpreting the results, and as described previously, concentrations can be different in these two types of nail samples, thus leading to an incorrect comparison between nails and hair. Finally, the most important condition for comparing nail and hair results is the selection of paired nails and hair samples from the same individuals, with a window of detection corresponding to the same time period. While it is difficult to compare samples corresponding to the same period of consumption, the comparison of nails and hair can be made for chronic consumers when the pattern of consumption has not changed in the last months before sample collection. In any case, considering the high interindividual variability in concentrations, the comparison of concentrations found in nail and hair samples taken from different individuals does not offer a reliable result.

5 | CONCLUSION

Although for some compounds a clear pattern was observed in terms of the matrix (hair, fingernails, or toenails) in which higher concentrations are detected, for the majority of the drugs, no conclusive results could be obtained. In any case, the number of publications comparing nails to hair is still limited, and in most cases, comparisons were made with a low number of paired samples. In addition, the lack of standardized sampling techniques, preanalytical procedures, and analytical methods for hair and nail analysis makes it difficult to compare the results of the available studies. Moreover, in most cases, the dosages taken by the sample donors were unknown, so, to date, a direct extrapolation of the amount of drug used cannot be performed based on concentrations found in nails or hair samples. Furthermore, choosing fingernails or toenails as the nail sample is important to highlight, because the contamination pathways and windows of detection reflected by these samples are different. Some of the aforementioned publications analyzed only one of these nail matrices or did not specify if fingernails or toenails were used in the study. This adds another variable to the interpretation of the results that should be considered in future studies. Nevertheless, nails are a promising matrix for the determination of long-term drug use, and of special interest as a substitute of hair analysis when this is not available, hence the need for further, well planned studies to characterize this matrix.

AUTHOR CONTRIBUTIONS

All authors contributed to the article conceptualization. Literature search and data analysis was performed by María Cobo Golpe under the supervision of Elena Lendoiro. Original draft was written by María Cobo Golpe and critically revised by Ana de-Castro-Ríos and Elena Lendoiro. All authors read and approved the final manuscript.

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