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Keratinous matrices for the assessment of drugs of abuse consumption: a correlation study between hair and nails

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Abstract

Keratinous matrices - hair and nails - accumulate substances over time and allow retrospective investigation of past consumption. Analysis of these matrices can provide information complementary to blood and urine analysis or can be used as standalone. So far, research has primarily focused on the detection of substances in hair, while studies in nails are scarce. In this study, we assessed concentrations of drugs of abuse and their metabolites in hair, finger- and toenails collected from the same individuals to evaluate differences and correlations between matrices. A total of 26 hair, 24 fingernail, and 18 toenail samples were collected. Samples were analyzed by a validated liquid chromatography-tandem mass spectrometry method able to simultaneously detect the following compounds: amphetamine (AMP), methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine, morphine (MOR), codeine (COD), 6-monoacetylmorphine (6-MAM), methadone (MTD), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), cocaine (COC), benzoylecgonine (BE), and ecgonine methyl ester (EME). Strong positive correlations between hair, finger- and toenails were present for COC, BE, EME, AMP and MDMA. MOR, COD, 6-MAM, MTD and EDDP showed positive trends. Concentrations were generally higher in nails compared to hair. Ratios between parent compounds and their metabolites were assessed for 6-MAM/MOR, EDDP/MTD, BE/COC and EME/COC. Preliminary cut-off concentrations for COC, BE, EME and AMP in finger- and toenails were proposed. In light of these results, nails can be considered as a useful alternative to hair for monitoring of long-term drug consumption. However, care should be taken regarding the variability in the accumulation of compounds between the matrices.

Keywords

Keratinous matrices, hair, nails, correlation, drugs of abuse, liquid chromatography tandem mass spectrometry

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Introduction

Keratinized matrices, such as hair and nails, are of considerable importance in forensic and clinical toxicology as they allow a stable accumulation of compounds over time. These matrices can thus provide long-term information about exposure to and consumption of substances, such as pharmaceuticals and illicit drugs [1, 2]. This is complementary to the more traditional matrices such as blood and urine, which have detection windows of hours to days [3]. Combined analysis of classical and keratinized matrices gives additional information about short-term (i.e. acute) and long-term (i.e. chronic) substance use. Furthermore, keratinized matrices can also be used for standalone analyses, when these relate to an event that occurred several weeks or months earlier, or when retrospective information over a longer period of time is required. Other advantages include the easy and non-invasive collection of samples, which does not require qualified medical staff nor specific conditions for transport and storage, and sample collection can be performed under close supervision to avoid adulteration.

Amphetamine-like stimulants, cocaine, and opioids continue to be in high demand and supply, with major impacts on public health [4]. According to the European Drug Report 2017, cocaine remains the most commonly used illicit stimulant in Europe [4]. The combined consumption of several drugs of abuse, i.e. polysubstance abuse, is highly prevalent and represents a significant health concern [5]. Due to drug-drug interactions, combinations of several drugs of abuse can significantly increase the risks of already harmful individual drugs. The monitoring of polysubstance abuse patients within a therapeutic or forensic/legal framework, represents a particular challenge that requires multi-analyte methods capable of detecting several compounds in a single run.

The importance of *hair* as a matrix for illicit drug testing has substantially increased in recent years, e.g., in driver's license regranting procedures and in investigations related to causes of death [6-9]. In this context, scientific organizations, such as the Society of Hair Testing (SoHT) and Substance Abuse and Mental Health Services Administration (SAMHSA), have published guidelines for hair analysis and recommendations for confirmatory cut-offs to identify use (Table 1) [10, 11]. On the other hand, studies on *nail* analysis for drugs of abuse are limited. Neither guidelines nor cut-off values are available for nail analysis, and therefore interpretation of results is often complicated. However, nail analysis can offer multiple advantages over hair analysis [1, 12]. First, the average growth rate of nails is substantially lower compared to hair (3 and 1 mm/month for finger- and

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toenails, respectively, compared to 1 cm/month for head hair); which would allow a more significant accumulation of substances [13, 14]. This can be relevant in situations where low concentrations are expected (e.g. low dosages). Secondly, while hair is characterized by a cyclic growth rate with different stages, nails grow at a constant rate, which facilitates the interpretation of results [15]. Hair strands that have been in a resting stage might result in the detection of more remoted consumption. Third, in contrast to hair, nails do not contain melanin. Since drug incorporation might be influenced by melanin concentrations, hair pigmentation becomes an important source of bias when interpreting detected drug concentrations [16, 17]. Fourth, nails can provide an alternative option in cases where hair is not (sufficiently) available (e.g., alopecia, newborns). Fifth, cosmetic hair treatments have been proven to reduce drug content in hair, which can be overcome through nail analysis [18, 19]. Possible disadvantages linked to the use of nails as matrix can be insufficient sample amount in cases of (finger)nail biting, presence of nail diseases (e.g. bacterial or fungal infections), and effects of nail polishing or cleaning. Also, drugs are incorporated into hair via the blood flow in the hair follicle, while incorporation into nails occurs by a dual mechanism of deposition into the root via blood flow in the nail matrix and the nail bed [1]. The vertical and horizontal incorporation into nails might complicate the interpretation of results.

Studies comparing concentrations of drugs of abuse in hair and nails are very scarce. Most include either a limited number of samples ($N < 9$; [20-22]) or encompass a limited number of compounds ($N \leq 3$; [23, 24]), and do not distinguish between finger- and toenails. To the best of our knowledge, only Shen et al. compared concentrations of opiates in hair and fingernails of 12 positive samples [25]. Except for morphine, they found significantly higher concentrations in hair than in nails and reported lower 6-monoacetylmorphine to morphine ratios in nails than in hair.

In the current study, we evaluated drug of abuse concentrations in paired hair and nail samples (both finger- and toenails) from individuals with a dependency to one or more drugs of abuse, and investigated the differences and correlations between both matrices. The investigated drugs of abuse and their metabolites, together with the recommended cut-off concentrations in hair, are presented in Table 1. The comparison between hair and nails can provide more information about the usefulness of nail analysis in forensic and clinical toxicology, which is especially relevant for those cases where hair is not available or not reliable (e.g. cosmetic treatment). Furthermore, by analysing both nails and hair, additional evidence can be gathered, and erroneous results and interpretations can be diminished.

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Material and methods

Reagents and materials

The following analytical standards were acquired from Cerilliant (Round Rock, TX, USA) as solutions at concentrations of 1.0 mg mL^{-1} in MeOH, unless otherwise stated: 6-monoacetylmorphine (6-MAM; in ACN), amphetamine (AMP), benzoylecgonine (BE), cocaine (COC; in ACN), codeine (COD; 100 µg mL^{-1}), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) perchlorate, ecgonine methyl ester (EME; in ACN), methamphetamine (mAMP), 3,4-methylenedioxyethylamphetamine (MDEA), 3,4-methylenedioxymethamphetamine (MDMA), and morphine (MOR). The methadone (MTD) standard was purchased as a solution of 1.0 mg mL^{-1} in MeOH from LGC GmbH (Luckenwalde, Germany). For each analyte, the corresponding deuterated analogue was included and used for quantification. The deuterated internal standards were obtained from Cerilliant as solutions in concentrations of 1.0 mg mL^{-1} in MeOH, unless otherwise stated: 6-MAM-D_3 (in ACN), AMP-D_8, BE-D_3, COC-D_3 (in ACN), COD-D_6 (100 µg mL^{-1}), EME-D_3 (100 µg mL^{-1} in ACN), MDEA-D_6 (100 µg mL^{-1}), MDMA-D_5, MTD-D_9, mAMP-D_{11} (100 µg mL^{-1}) and MOR-D_3. The deuterated internal standard EDDP-D_3 perchlorate was bought from LGC GmbH as a solution of 1.0 mg mL^{-1}. Starting from the purchased stock solutions, working solutions for both standards and internal standards were prepared in order to contain all analytes in the same concentrations. Three standard working solutions were prepared in MeOH at concentrations of 1 ng μL^{-1}, 100 pg μL^{-1}, and 10 pg μL^{-1}. The internal standard working solution was prepared in MeOH at a concentration 100 pg μL^{-1}. All solutions were stored at -20 °C.

Sample collection

Hair and nail samples were collected from inpatients engaged in a treatment program for substance use disorders at the Psychiatric Centre Multiversum together with an informed consent. All patients met DSM-5 criteria for substance use disorders [26]. The Ethical Committee of the University Hospital of Antwerp (UZA) approved the study (Belgian registration number B30020169233). Hair samples were collected from the vertex posterior region of the head and cut as closely to the scalp as possible. The first 3 cm segment from the proximal end was used for further analysis. Nail samples were obtained by clipping approximately 1-2 mm of the distal edges of all ten finger- and toenails. Finger- and toenail samples were collected and analyzed separately. Samples were stored in aluminium foil at room temperature until analysis.

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Data on drug consumption and cosmetic treatment

Together with hair and nail samples, a detailed anamnesis of past drug consumption was taken. Patients were asked about their drug consumption in the past year using an adapted version of the Timeline Follow-back (TLFB) method [27]. Per subject, the amount of drug consumed in the past year was used to calculate an average weekly consumption.

Patients were asked whether they had bleached, dyed, permed or thermally straightened their hair in the past year, as cosmetic hair treatment may lead to a degradation or removal of drugs in hair [18, 19]. Nail polishing, the use of acetone and other nail treatments were also recorded. Patients reporting any cosmetic treatment of the hair or nails were excluded from the study.

Sample preparation and instrumental analysis

The sample preparation and the liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) analysis were performed according to a previously validated method [28]. Briefly, hair and nail samples were subsequently decontaminated, pulverized, extracted and filtered. Finally, the extracts were analysed on an LC-system (Agilent Infinity 1200, Diegem, Belgium) coupled to a triple quadrupole mass spectrometer (Agilent 6410 MS).

The accuracy and the analytical performance of the method were assessed and controlled by means of successful participation to proficiency tests organized by Arvecon GmbH and the SoHT. Furthermore, each batch included the analysis of a certified authentic hair sample containing common drugs of abuse as well as the verification of the retention time and the qualifier/quantifier ratios.

Data analysis

Statistical analysis was performed using R (version 3.3.1., The R Foundation for Statistical Computing). Due to non-normality of the data (tested through histograms and QQ-plots of the raw data), further analyses were performed with non-parametric statistics. Differences between matrix concentrations and metabolite to parent compound ratios between matrices were assessed using Wilcoxon rank sum test. Associations between hair and nails (finger- and toenails) were expressed using Spearman correlation. Using the regression coefficients $\beta 0$ and $\beta 1$, the existing cut-off concentrations for COC, BE, EME and AMP in hair [10] were translated to preliminary cut-off concentrations in finger- and toenails. For all statistical tests, a p-value < 0.05 was considered statistically significant. Results are presented as mean ± standard deviation (SD) or as median [interquartile range (IQR)].

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Results

A total number of 26 patients engaged in treatment for substance use disorders were included in the study. Four participants were female and 22 participants were male, aged 22 to 59 with a mean age of 36 ± 8.5 years. Sixteen patients provided hair, finger- and toenail samples, 8 patients provided only hair and fingernail samples, and 2 patients provided only hair and toenail samples.

Table 2 shows the number of positive samples, the concentration ranges and the median [IQR] concentration for each analyte. Differences in concentrations between matrices and correlations were statistically assessed for COC, BE, EME, AMP, MDMA (see Table S1 in the supplementary material), whilst for MOR, COD, 6-MAM, MTD, and EDDP, only visual examinations were performed due to the limited number of positive samples available. No significant difference in concentrations measured in the various matrices collected from the same individuals could be observed. Only for COC, concentrations in toenails were significantly lower compared to the other two matrices. Although the differences in concentrations between matrices were not statistically significant, some trends could be observed. Except for COC, compound concentrations were higher in nails (finger- or toenails) compared to hair. COC concentrations were higher in hair, followed by finger- and then toenails. For BE, EME, MOR, 6-MAM and EDDP, concentrations were highest in fingernails, whilst for AMP, MDMA, COD and MTD, concentrations were highest in toenails. Between matrices correlations were investigated for each compound. All Spearman correlations were significant and positive with ρ ranging from 0.75 to 0.97, except for MDMA (correlation of hair versus fingernails and hair versus toenails were positive but not significant) (Table S1). In Figures 1 and 2 the different correlations for COC, BE, EME, AMP and MDMA are represented. The existing cut-off concentrations for hair analysis in combination with the regression coefficients $\beta 0$ and $\beta 1$ of the hair versus fingernails and hair versus toenails correlations for COC, BE, EME and AMP (Table S1) were used to make some preliminary predictions of cut-off concentrations for finger- and toenail analysis (Table 3). As visualized in Figure 3, there are positive relationships between the different matrices for MOR, COD, 6-MAM, MTD and EDDP.

In addition, the ratios of metabolite versus parent compound were calculated for 6-MAM/MOR, EDDP/MTD, BE/COC and EME/COC in all matrices. Due to the limited number of samples for 6-MAM/MOR and EDDP/MTD, only the ratios BE/COC and EME/COC were assessed statistically. Median ratios for 6-MAM/MOR were 1.06, 3.35 and

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0.92 in hair, finger- and toenails, respectively. For EDDP/MTD, median ratios were 0.063, 0.19 and 0.063 in hair, finger- and toenails, respectively. Median ratios for BE/COC were 0.21, 0.90 and 1.87 in hair, finger- and toenails, respectively. Differences in BE/COC ratios between matrices were significant (p-value < 0.001 for hair versus fingernails and hair versus toenails, p-value = 0.006 for finger- versus toenails). Figure 4 displays the boxplots of BE/COC ratios in the different matrices. For EME/COC median ratios were 0.019, 0.033 and 0.068 in hair, finger- and toenails, respectively. Differences in EME/COC ratios between matrices were significant for hair versus fingernails and hair versus toenails (p-value = 0.02 and p-value = 0.003, respectively). For finger- versus toenails the difference in EME/COC ratios was not significant (p-value = 0.2). The boxplots of EME/COC ratios in the different matrices are visualized in Figure 5.

Tables S2, S3, S4 and S5 in the supplementary material, show a comparison between the average weekly consumption as reported using the TLFB method and the concentrations in hair, finger- and toenails for each subject. In terms of reported substances versus detected substances, there was a good agreement between the TLFB method and hair and nails with regards to declared use of substances and detection in the keratinous matrices (positive or negative). The agreement between the TLFB method and hair and nails in terms of amounts reported and concentrations measured in hair and nails was not that clear. In general, high reported use corresponded to high hair, finger- and toenail concentrations. However, some patients reporting limited use still had high concentrations in both hair and nails.

Discussion

Significant positive correlations between hair and nails (both finger- and toenails) and between finger- and toenails were present for COC, BE, EME and AMP (Figures 1 and 2). For MDMA, correlations were positive, but only the correlation between finger- and toenails was significant (Figure 2), probably due to the limited number of positive MDMA samples (N = 9). For MOR, COD, 6-MAM, MTD, and EDDP, positive relationships could be observed but these could not be investigated statistically due to the limited number of samples (N < 9, Figure 3). The presence of these positive trends indicates that finger- or toenails can be used as alternative matrices to hair.

Compound concentrations were generally higher in nails (finger- and toenails) than in hair, except for COC. Observing high concentrations in nails could be linked to the slower growth rate of finger- and toenails compared to hair (3 and 1 mm/month versus 1 cm/month, respectively) which might result in a higher accumulation of compounds in nails [13]. The

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slower growth rate could explain why for AMP, COD, and MTD, highest concentrations were measured in toenails, followed by fingernails and then by hair. However, other factors must be taken into consideration when comparing concentrations between matrices. First, the different mechanisms of incorporation into hair or nails may influence the observed concentrations. In fact, blood supply through the hair follicle is the main route for hair [2], while for nails compounds incorporate through a dual mechanism of deposition into the root via blood circulation in the nail matrix and bed [1]. Although the influence of the vertical incorporation is limited (approximately 20%), this vertical and horizontal incorporation into nails might complicate the interpretation of results. Second, unlike hair which is characterized by a cyclic growth rate with different stages [14], nails are permanently formed and do not undergo resting growth stages [15]. The cyclic growth rate of hair can sometimes complicate the interpretation of the obtained results, as it might result in the detection of past use in hair strands that are in resting stage and thus have not been growing. However, as the number of hair stands in resting stage is rather small (approximately 15%), especially at the vertex posterior region where hair samples are taken, the impact of this factor is likely limited [14]. Third, the physicochemical properties of drugs might influence the extend of incorporation into hair and nails. In fact, molecules have to diffuse from the systemic blood circulation across the cell membrane of the growing cells at the hair follicle or the nail matrix and the nail bed. The rate of diffusion is related to the lipid solubility of the compound, and the pH gradient between the plasma and the cell. In hair, it has been shown that the pH gradient from plasma, pH = 7.3, to more acidic conditions, within the melanocytes/ keratinocytes (pH 3-6) favours the incorporation of basic drugs over acidic drugs [2, 14]. This was also observed by Kuwayama and colleagues, who reported higher concentrations of basic compounds in hair compared to toenails, whereas concentrations of weakly acidic and neutral compounds tended to be higher in toenails than in hair [29]. The physicochemical properties of a drug are also likely to influence the amount of incorporation through sweat and sebum and will be more important for neutral and lipophilic drugs.

The higher concentrations of COC present in hair compared to nails might be explained by the absence of melanin in nails in contrast to hair. Melanin is known to bind several compounds in hair and the affinity of a compound to melanin depends on the physicochemical properties of the compound. Incorporation of more basic drugs into hair has shown a positive relationship to melanin [16, 17]. The possible influence of melanin was also acknowledged by Shen et al., which attributed the detection of higher acetylcodeine, COD and 6-MAM in hair to the black hair of subjects [25]. For some compounds, the higher

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affinity to melanin might counterbalance or even surpass the higher accumulation due to the slower growth rate of nails, and, thus result in higher concentrations in hair than in nails as observed for COC. The influence of melanin might also explain why for EME, MOR and MDMA, the second highest concentrations are found in hair.

An important consequence of the observed differences in concentrations between hair, finger- and toenails is that cut-off concentrations established for hair are not valid to interpret concentrations measured in nails. Therefore, for the compounds COC, BE, EME and AMP, some preliminary cut-off concentrations for finger- and toenails are proposed (Table 3). To determine these preliminary cut-off concentrations, the regression line was calculated using nail concentrations as predicted variable and hair concentrations as predictor. The cut-off concentrations in hair were then fed to the equation to estimate the corresponding y-values (predicted cut-off concentrations in nails). As COC concentrations are higher in hair compared to finger- and toenails, the preliminary cut-off concentrations are lower in finger- and toenails. For AMP, the concentrations are lower in hair compared to finger- and toenails, and thus the preliminary cut-off concentrations are higher in finger- and toenails. There is an absolute need to update or confirm these proposed cut-offs in future studies with a larger population.

The amount and type of external contamination varies between hair, finger- and toenails. Fingernails can be easily externally contaminated with drug residues, while external contamination from sweat is more probable for toenails. Hair is more likely to be contaminated by vapors from smoked drugs. The influence of external contamination on the compound concentration should be minimal as several strategies are used to reduce it. First, a decontamination step is performed before analysis in order to eliminate external contaminants and leave only the embedded substances. A second strategy is the detection of metabolites as indicators of human metabolism in addition to the parent compounds. For example, the presence of 6-MAM is required if MOR is present, and the presence of BE is required if COC is present [10, 11]. Third, the determination of metabolite to parent drug ratios is also recommended for discrimination between drug intake and external contamination. In hair, the SAMHSA recommends a ratio of BE/COC greater than 5%. Ratios between parent compounds and their respective metabolites also give information on the differences in accumulation profiles between hair and nails. Results obtained showed that the ratios were different between hair, finger- and toenails. In hair, median COC concentrations were higher than median BE concentrations, while in nails median BE concentrations were higher than median COC concentrations. As a result, BE/COC ratios were significantly higher in finger-

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and toenails than in hair, with the highest ratio present in toenails (Figure 4). This finding was also reported by Krumbiegel et al. in three post-mortem cases where they found higher COC than BE concentrations in hair, but higher BE than COC concentrations in nails [20]. EME/COC ratios were also highest in toenails followed by fingernails and then hair (Figure 5). 6-MAM/MOR and EDDP/MTD ratios were highest in fingernails and similar in hair and toenails. However, as only limited amount of positive samples were available for 6-MAM, MOR, EDDP and MTD, these findings need to be confirmed. Results obtained so far indicate that the ratios are different in hair, finger- and toenails and this should be taken into consideration when using the ratios to discriminate between external contamination and drug intake.

There was a good agreement between patients reporting the consumption of a drug of abuse, and the presence of this substance in the hair, finger- and toenails of the patient. In addition, patients reporting high consumption of drugs, also had high concentrations in their hair and nails. Still, some patients reporting relatively low consumption showed relatively high concentrations in both hair and nails. In these cases, one might question the reliability of the answers given by the patients. Although patients were encouraged to report their true consumption, some of them might have reported an underestimation of their effective consumption.

Hair grows at a rate of 1 cm per month [14], therefore, the 3 cm proximal segment corresponds to 3 months of growth and consumption of drugs in the last 3 months. On the other hand, finger- and toenails grow at a rate of 1 mm and 0.3 mm per month, respectively . Moreover, as the 1-2 mm clippings were taken from the distal edges of the nails, the regeneration time (i.e., the time to grow from the nail matrix to the nail's free edge) of the nail must be taken into account. The regeneration time is 3–5 and 8–16 months for finger- and toenails, respectively [15]. Thus, the 1-2 mm fingernail clippings correspond to 1-2 months of growth 3-5 months ago, while the 1-2 mm toenail clippings correspond to 6-9 months of growth 8-16 months ago. As a result, the collected hair, finger- and toenail segments did not represent the same period of time. However, the samples in this study were collected from patients with a diagnosed and long-lasting addiction problem, which reported stable drug consumption patterns. This could be deduced from the TLFBs that were collected from these patients; in these questionnaires patients reported a stable consumption for a period of one to several years prior to sample collection.

To the best of our knowledge, this is the first article that systematically compares and statistically investigates concentrations of drugs of abuse in hair, finger and toenails obtained

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from the same subjects. Future studies with a higher number of positive samples, especially for MDMA, MDEA, mAMP, MOR, COD, 6-MAM, MTD and EDDP, are needed to confirm and elaborate on the current results. However, results obtained so far show that nails are a valuable alternative or complement to hair as positive correlations and relationships are present between them. At the same time, this study underlines the need for specific cut-off concentrations for nail analysis as they exist for hair.

Conclusions

Today, the detection of drugs of abuse in hair samples is used in routine laboratories to evaluate long-term drug consumption. This study shows that COC, BE, EME, AMP and MDMA concentrations in hair and nails (both finger- and toenails) are significantly and positively correlated. For MOR, COD, 6-MAM, MTD, and EDDP, positive relationships are present between hair and nails. The differences in parent compound to metabolite ratios (BE/COC, EME/COC, 6-MAM/MOR and EDDP/MTD) can indicate important variances in accumulation of compounds between hair, finger- and toenails. In summary, nails are a useful alternative to hair for monitoring long-term drugs of abuse consumption. However, care should be taken regarding the variability in the accumulation of compounds between the matrices. The article proposes some preliminary cut-off values for finger- and toenail analysis, but future studies are needed confirm these.

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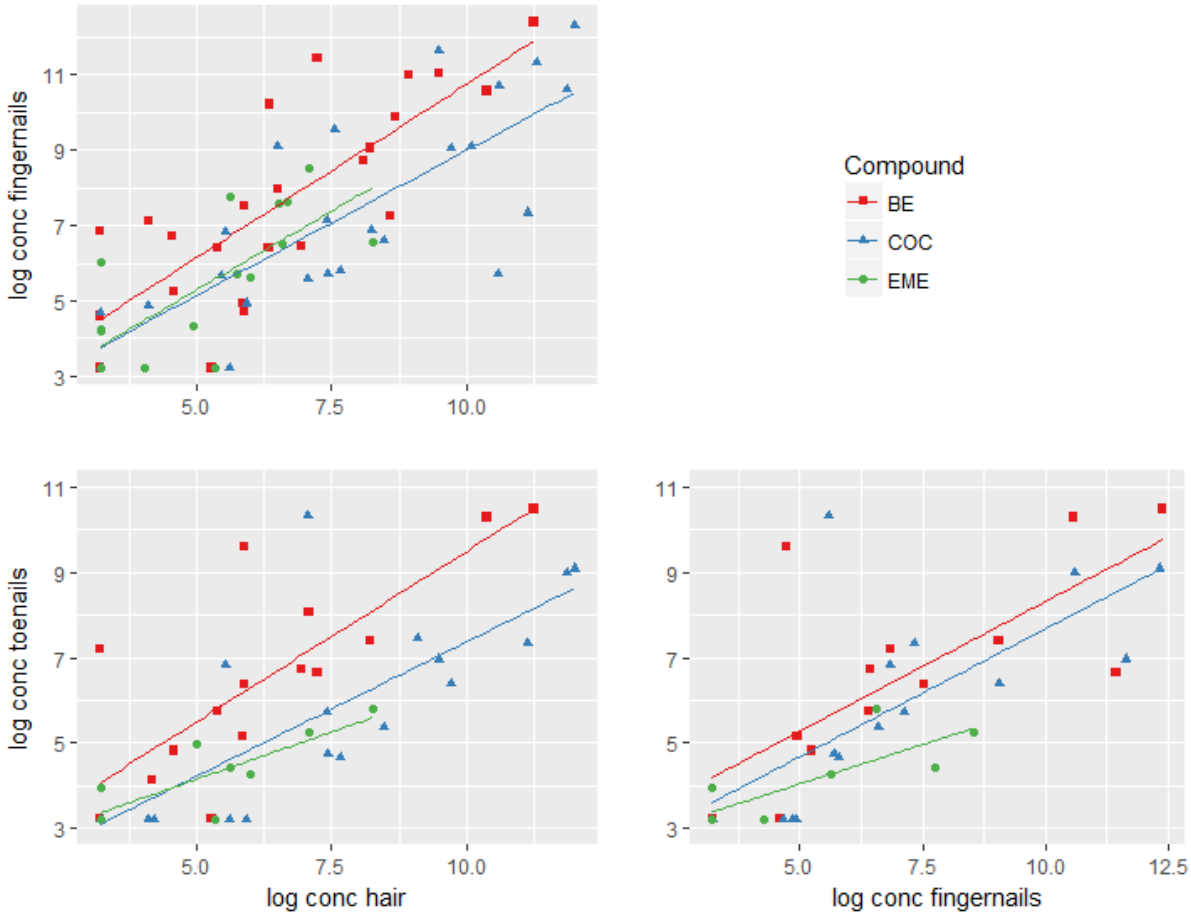

Figure 1. Correlations for benzoylecgonine (BE), cocaine (COC) and ecgonine methyl ester (EME) concentrations between hair, finger- and toenail samples. Concentrations were log-transformed.

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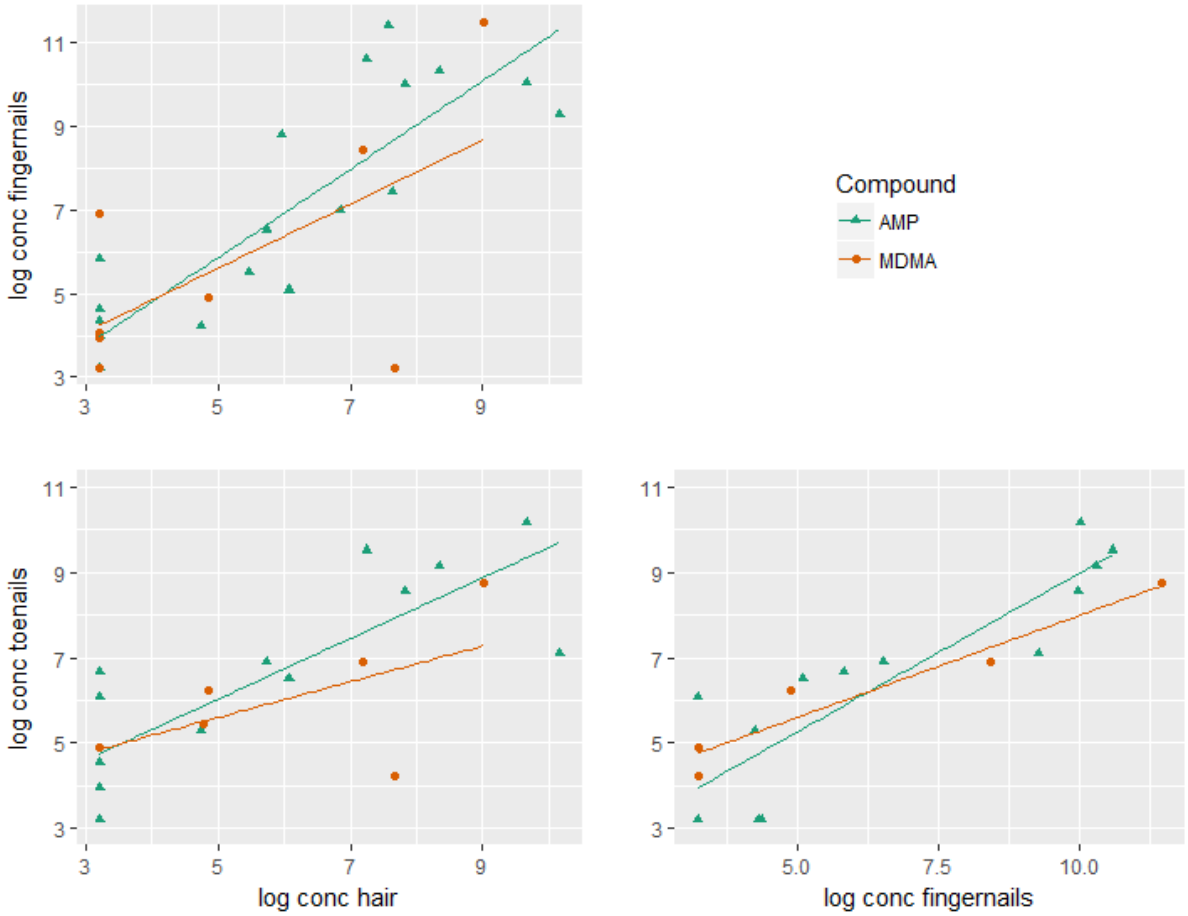

Figure 2. Correlations for amphetamine (AMP) and 3,4-methylenedioxymethamphetamine (MDMA) concentrations between hair, finger- and toenail samples. Concentrations were log-transformed.

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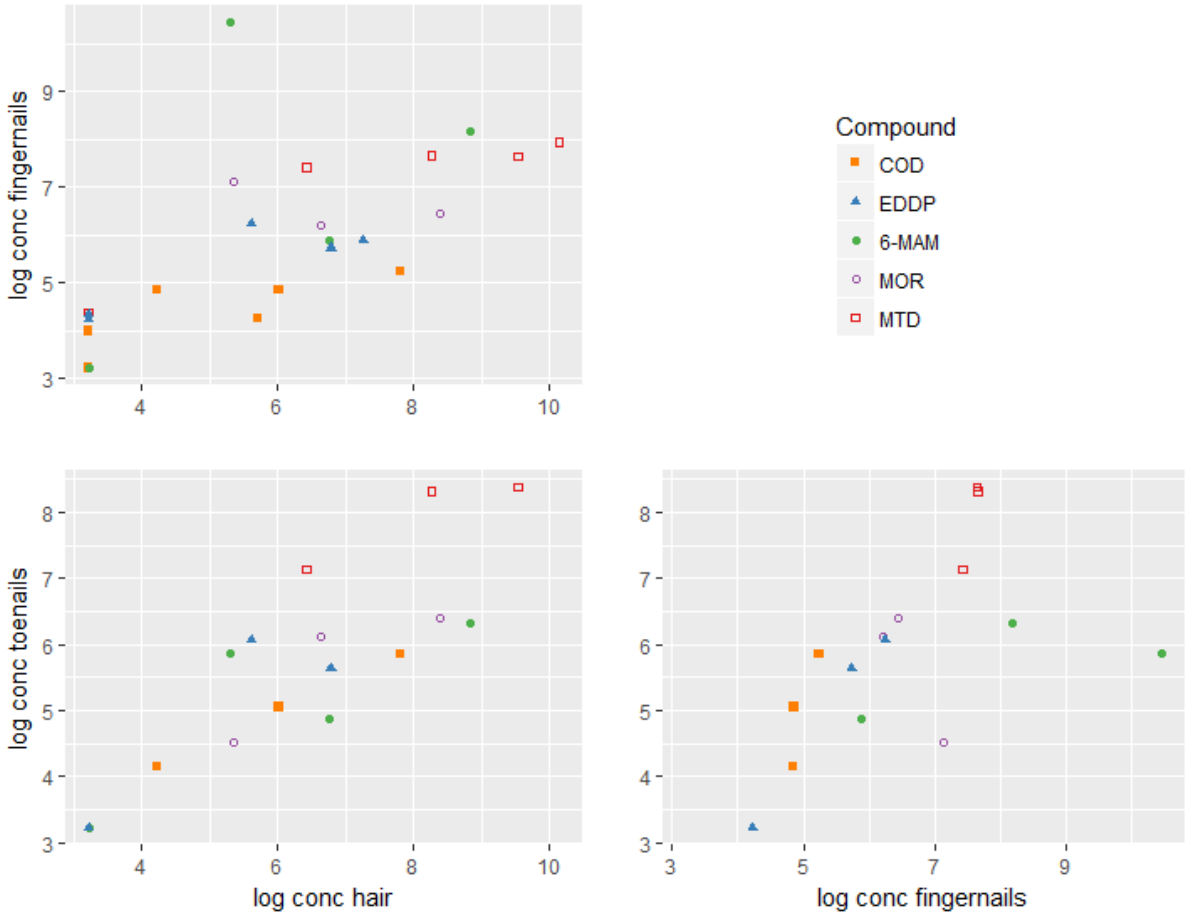

Figure 3. Scatter plots for codeine (COD), ethylidene dimethyldiphenyl pyrrolidine (EDDP), 6-monoacetylmorphine (6-MAM), morphine (MOR) and methadone (MTD) concentrations between hair, finger- and toenail samples. Concentrations were log-transformed.

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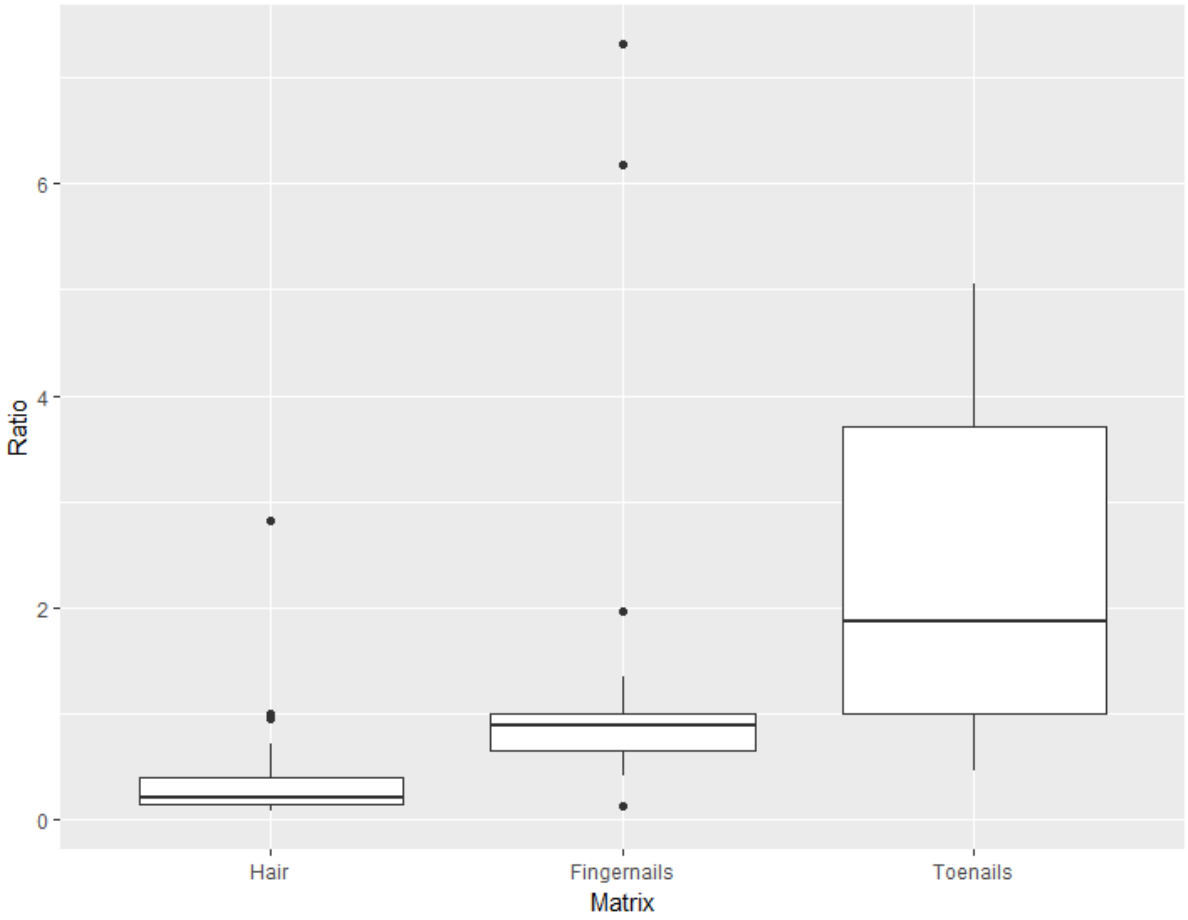

Figure 4. Boxplots comparing the benzoylecgonine/cocaine ratios in hair, finger- and toenails.

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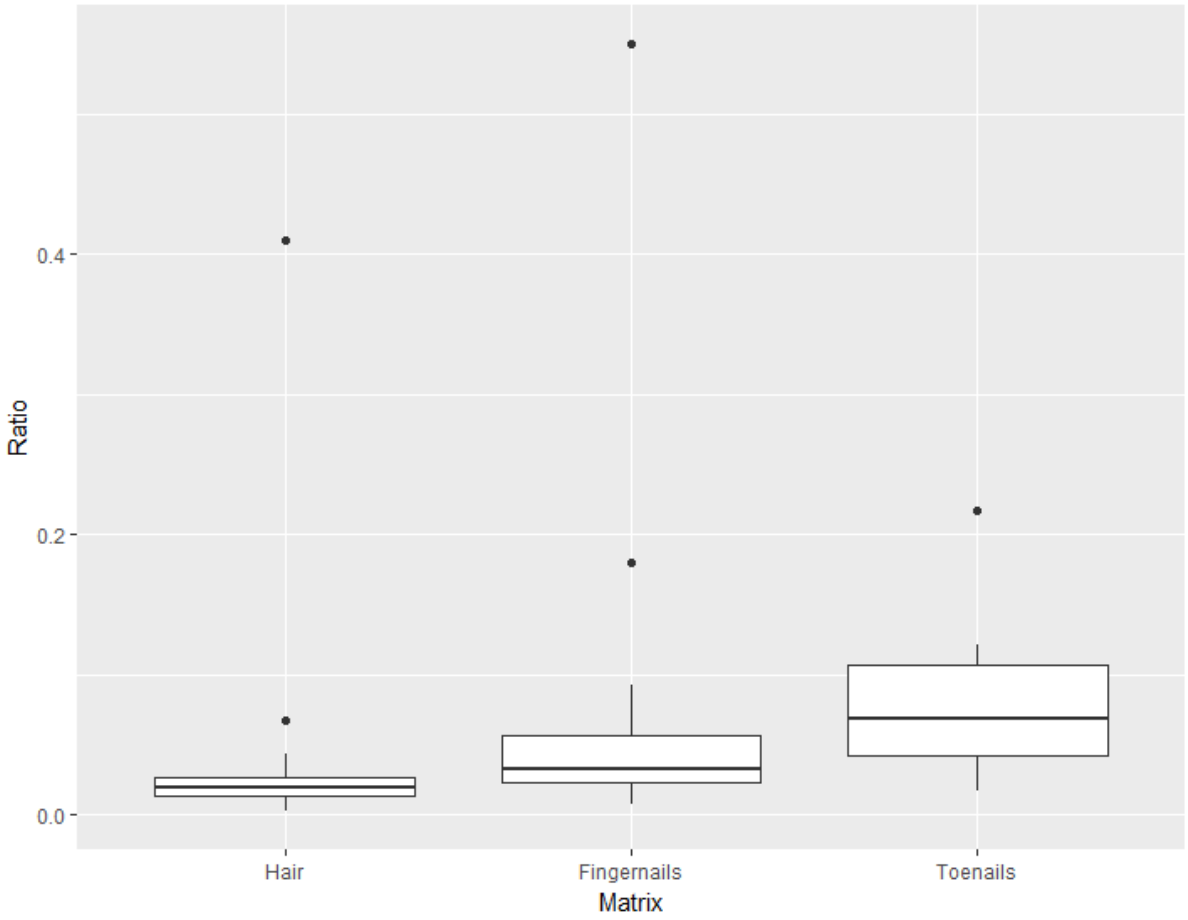

Figure 5. Boxplots comparing the ecgonine methyl ester/cocaine ratios in hair, finger- and toenails.

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Table 1. Overview of the eight drugs of abuse (indicated in bold), their metabolites, and the corresponding cut-off concentrations.

Compound	Abbreviation	Recommended cutoff concentrations in hair (pg/ mg)		Notes
		SoHT	SAMHSA	
Amphetamine-type stimulants				
Amphetamine	AMP	200	300	
Methamphetamine	mAMP	200	300	
3,4-methylenedioxymethamphetamine	MDMA	200	300	
3,4-methylenedioxyethylamphetamine	MDEA	200	300	
Opioids				
Morphine	MOR	200	200	Parent compound, but also metabolite of **heroin** and **codeine**
Codeine	COD	200	200	
6-Monoacetylmorphine	6-MAM	200	200	Minor but exclusive metabolite of **heroin**
Methadone	MTD	200	/	
2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine	EDDP	50	/	Metabolite of **methadone**
Cocaine				
Cocaine	COC	500	500	
Benzoylecgonine	BE	50	> 5% COC level	Metabolite of **cocaine**
Ecgonine methyl ester	EME	50	/	Metabolite of **cocaine**

Abbreviations: SAMHSA, Substance Abuse and Mental Health Services Administration; SoHT, Society of Hair Testing

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Table 2. The number of positive samples, the concentration ranges and the median [interquartile range (IQR)] concentration for each drug of abuse. Results of methamphetamine are not shown since there was only one positive sample.

Compound	Matrix	Nr. of positive cases	Conc. range (pg/mg)[a]	Median [IQR] (pg/mg)
COC	Hair	26	25 - 163420	2011 [298; 22304]
	Fingernails	24	25 - 224085	942 [284; 10246]
	Toenails	18	25 - 30802	260 [25; 1423]
BE	Hair	26	25 - 76924	566 [123; 3621]
	Fingernails	24	25 - 242124	1311 [499; 21645]
	Toenails	18	25 - 35985	676 [109; 2024]
EME	Hair	19	25 - 3837	178 [25; 607]
	Fingernails	18	25 - 5059	179 [32; 697]
	Toenails	12	25 - 325	63 [25; 129]
AMP	Hair	26	25 - 25999	189 [25; 1540]
	Fingernails	23	25 - 87131	244 [61; 8604]
	Toenails	19	25 - 25726	552 [45; 2194]
MDMA	Hair	9	25 - 8298	119 [25; 1325]
	Fingernails	8	25 - 95411	95 [45; 1875]
	Toenails	6	68- 6461	368 [156; 868]
MDEA	Hair	2	25 - 50	38 [31; 44]
	Fingernails	2	72 - 258	165 [119; 212]
	Toenails	2	25 - 50	38 [31; 44]
MOR	Hair	4	25 - 4446	485 [166; 1679]
	Fingernails	4	25 - 1253	562 [379; 784]
	Toenails	3	91 - 604	456 [274; 530]
COD	Hair	7	25 - 2467	82 [47; 355]
	Fingernails	6	25 189	99 [58; 129]
	Toenails	4	64 - 346	156 [110; 251]
6-MAM	Hair	4	25 - 6916	202 [25; 855]
	Fingernails	4	25 - 34665	1966 [25; 11344]
	Toenails	3	25 - 557	244 [104; 406]
MTD	Hair	5	25 - 25518	389 [617; 13900]
	Fingernails	5	80 - 2835	2092 [1668; 2112]
	Toenails	4	80 - 4353	4073 [2663; 4213]
EDDP	Hair	5	25 - 1420	274 [25; 883]
	Fingernails	5	68- 517	308 [77; 358]
	Toenails	4	25 - 433	279 [152; 356]

[a] Positive samples with concentrations lower than the LLOQ (50 pg/mg) were reported as 25 pg/mg (LLOQ/2)

Abbreviations: 6-MAM, 6-monoacetylmorphine; AMP, amphetamine; BE, benzoylecgonine; COC, cocaine; COD, codeine; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; EME, ecgonine methyl ester; LLOQ, lower limit of quantification; MDEA, 3,4-methylenedioxyethylamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MOR, morphine; MTD, methadone

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Table 3. Preliminary predictions of cut-off values for finger- and toenail analysis.

Compound	SoHT cut-off value in hair (pg/mg)	Predicted cut-off value in fingernails (pg/mg)	Predicted cut-off value in toenails (pg/mg)
COC	500	440	150
BE	50	175	105
EME	50	80	40
AMP	200	485	505

Abbreviations: AMP, amphetamine; BE, benzoylecgonine; COC, cocaine; EME, ecgonine methyl ester, SoHT, Society of Hair Testing

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