To the Editor:

Crack cocaine or smoked cocaine addiction represents a social problem in numerous countries. The consumption becomes rapidly chronic in almost all consumers, who spend time and money to find their drug. This chronic intoxication results in psychic disturbances that may lead to asocial and aggressive behavior. For clinical, legal, and epidemiologic purposes it is interesting to be able to document long-term addiction. The detection of the anhydroecgonine methylester (AEME) marker of cocaine pyrolysis in hair has been reported to characterize chronic consumption (1). Because the collection of hair is not always possible, nail clippings could represent an interesting alternative to test for AEME in addictive patients.

All solvents and reagents were high-performance liquid chromatography grade and were purchased from Merck (Darmstadt, Germany). Cocaine (COC), benzoylecgonine (BZE), ecgonine methylester (EME), AEME, and their deuterated analogues were purchased from Promochem (Molsheim, France). The N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) plus 1% trimethylchlorosilane (TMCS) was purchased from Interchim (Montluçon, France).

Two men (20 and 25 years old) were arrested two days after a murder. The judge in charge of the case requested a toxicological screening focused on drugs of abuse. Because of the delay, only urine was sampled. To document long-term exposure, nails were collected in absence of suitable hair (subjects were both totally shaved).

For each of the subjects, a sample of 100 mg of nails was collected by clipping the nails of both hands, leading to pieces of 2 to 3 mm. The nail clippings from both hands were mixed and stored in a sterile box.

Prior to analysis, decontamination was achieved by hot water then dichloromethane baths (5 at least) followed by successive drying in order to eliminate any risk of contamination by a contact with the drug or its smoke. Then the dried nails were finely pulverized in a bullet blender and weighed. Specimens (50 mg) of pulverized nails and 200 ng of deuterated cocaine and metabolites as internal standards: EME-d3, COC-d3, CE-d3, and BZE-d3 were incubated at 56°C overnight in 1 mL 0.1M HCl.

Centrifuged urine (1 mL) spiked with 200 ng of deuterated cocaine and metabolites (EME-d3, COC-d3, CE-d3, and BZE-d3) as internal standards and hydrolyzed nails prepared according to the described procedure were extracted with 10 mL chloroform/isopropanol/n-heptane (50:17:33, v/v/v) under alkaline conditions (2 mL 1M phosphate buffer at pH 8.4). After agitation and centrifugation, the organic phase was purified by an additional acid extraction (5 mL of 0.2M HCl), and the aqueous layer was re-extracted with 2 mL phosphate buffer, 1 mL 1M NaOH, and 5 mL chloroform. After agitation and centrifugation, the organic phase was removed and evaporated to dryness at 45°C in a Speed Vac concentrator. Then BSTFA +1% TMCS (35 μL) was added to the dry extract, which was sealed and heated at 70°C for 20 min. A 1.0-μL portion of the derived extract was injected into the gas chromatograph (GC) column through an Agilent (Palo Alto, CA) 7673 autosampler.

The analysis was carried out on a GC (6890) coupled to a mass selective detector (MSD) (5973) from Agilent Technologies. The capillary column (5% phenyl-95% methyl siloxane: 30-m x 0.25-mm i.d.) was purchased from Machrey Nagel; the flow rate of the carrier gas (helium, purity grade N 55) was 1.0 mL/min. The MSD was operated at 70 eV, had an ion source temperature of 230°C, and was autotuned dialy with perfluorotributylamine. The electron multiplier voltage was set at 300 V above the autotune voltage.

The column oven temperature was programmed from an initial temperature of 60°C to 295°C at 30°C/min and maintained at 295°C for the final 10 min. Splitless injection with a purge time of 2 min was employed. The injector temperature was 260°C (1).

Table 1. Selected Ions and Retention Times

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Ions* (m/z)</th>
<th>Ions of Internal standard d3 (m/z)</th>
<th>Retention Times (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEME</td>
<td>152, 166, 181</td>
<td>99, 85, 274</td>
<td>5.95</td>
</tr>
<tr>
<td>EME</td>
<td>96, 82, 271</td>
<td>99, 85, 274</td>
<td>7.07</td>
</tr>
<tr>
<td>COC</td>
<td>182, 82, 303</td>
<td>185, 85, 306</td>
<td>9.09</td>
</tr>
<tr>
<td>CE</td>
<td>196, 82, 317</td>
<td>199, 85, 320</td>
<td>9.26</td>
</tr>
<tr>
<td>BZE</td>
<td>240, 82, 361</td>
<td>243, 85, 364</td>
<td>9.30</td>
</tr>
</tbody>
</table>

* Ions in bold were used for quantitation.

Abbreviations: AEME: anhydroecgonine methylester; EME: ecgonine methylester; COC: cocaine; CE: cocaethylene; BZE: benzoylecgonine.
The MSD was operated in selected ion monitoring mode. Table I shows the ions monitored for AEME, COC, EME, CE, and internal standards (COC-d3, BZE-d3, EME, and CE-d3) and the retention times. Ratios of drug integrated ion peak area versus the integrated relative internal standard ion peak area were used for quantification. AEME was quantified by comparing the ratio of peak areas with those of deuterated COC because of the unavailability of deuterated AEME. The limit of detection (LOD) of COC and metabolites extracted from urine was approximately 1 ng/mL for all analytes (signal-to-noise ratio greater than 3). For hair and nails, the LODs were 0.10 ng/mg AEME, 0.50 ng/mg EME, 0.050 ng/mg COC, 0.050 ng/mg CE, and 0.15 ng/mg BZE.

The analysis of the nails samples from both subjects (1 and 2) leads to the detection of COC and its major metabolites, BE and EME (Figure 1). The presence of EME is indicative of nails’ incorporation via the blood stream rather than a result of external contamination. This is not the case for BZE, which can be a possible product of non-enzymatic hydrolysis of cocaine. The mass spectrum of the 5.95 nm peak provided a 99% match to an AEME library standard as shown on Figure 2. This is a significant marker of pyrolysis of cocaine, but it can be an external contaminant. However, the concentrations of COC and relative compounds of the dichloromethane baths were lower than three times the signal-to-noise ratio after the fifth bath, avoiding any risk of a false-positive result (Figure 3). The analysis of toenails and/or the comparison of analysis of right and left hand would have been of interest, but these samples were not collected. Urinary concentrations presented in Table II are associated to nails results. For one of the patients, AEME was lower than the LOD in urine. In the absence of hair sample, crack cocaine use was therefore established by nail analysis.

The level of concentrations of COC and metabolites in nails allows to expect that their analysis should be possible with less than 50 mg of specimen. As in hair, COC was present in nail at higher concentration when compared with the other analytes. Tropacocaine, pseudococaine, and cinnamoylcocaine were also found in nails, reflecting the consumption of a cocaine-based paste (pasta) or crack prepared from badly purified cocaine. These alkaloids were not detected in urine samples.

AEME, an analytical marker of crack use not easily detected in blood samples, can be found in urine, saliva, sweat, and hair.
However, urine should be collected quickly, and it can be lacking in cases of emergency or death. AEME tests positive within a few hours in saliva and sweat, but collection of these specimens is not usual and cannot be done postmortem. Hair, which is the best specimen for this type of investigation, can be very short or even shaven. In these cases, the analysis of nails could be an interesting alternative. Although the incorporation of COC and metabolites had been extensively investigated for hair, very few reports are available for fingernails or toenails, another keratinized matrix. The use of this sample can be an alternative or a complement to hair specimens in the detection and quantitation of long-term drug use. The mechanism of drug nail incorporation is complex and multifaceted. In fact, it depends not only on the drug consumption but also on the physico-chemical properties of the analyte, and in part on external contamination (3-5). The mechanism of drug incorporation into nail includes rapid diffusion from the skin under the nail plate, incorporation of newly formed matrix cells containing drug, and transfer from sebum and sweat (1). Engelhart and Jenkins (5) showed that external contamination of nails for COC and opiates is not a significant factor.

The time profiles of drug disposition into nails is similar to hair with drug concentration appearing within three days after the last drug administration, peaking within 2-3 weeks of drug administration, and decreasing approximately four weeks post administration (4,6). The nails can be collected by scraping the ventral surface (4) or by clipping. Clipping represent specimens of late exposure: between three months to six months growth before collection (7). Indeed, nails grows at an average rate of less than 0.1 cm per week, and numerous factors such age, gender, race, and season may influence their growth (3,8).

Detection of COC and metabolites in toe- or fingernails has been described in postmortem cases (5,8-10) and their disposition following subcutaneous injection extensively studied by Ropero-Miller et al. (4). The detection of crack cocaine marker AEME in toe and fingernails was first described by Garside et al. (11).

Our report describes the use of this specimen for detection of past crack use in a case of crime history. This could be essential to appreciate the impact of the drug on the behavior or to study these pathological findings.

The major problem of nails analysis in the case of crack cocaine use is the potential external contamination of AEME, produced by pyrolysis during smoking or handling. However, external incorporation seems to be marginal (5) and contamination can be solved by multiple washes before doing the analysis. The combined wash fractions of COC and metabolites concentrations was not calculated in our study, but Ropero-Miller et al. (4) report that nail scraping concentration represent only 5 to 40% of the combined wash fractions measured. It appears that drug may absorb closer to the nail surface than in hair surface and that they can be removed more easily than in hair by washes procedures (4). Our procedure, yet to be used for hair, allows an effective decontamination. Then nails can be easily blended and treated as other biological samples. Despite no clear relationship between nail drug concentration and dose (4,5), detection of AEME in nail specimen seems an appropriate tool for the detection of past drug use.

The identification of the specific marker of smoked cocaine in nail shows that nail specimen is an alternative to hair to document crack cocaine chronic consumption. The carefully decontamination of the sample prevents the risk of external contamination, which is the main source of error for the interpretation.

Fingernail clippings obtained from two subjects suspected of smoking cocaine use were quantitatively analyzed for the presence of AEME, the analytical marker of crack. Prior to analysis, decontamination was performed with hot water and dichloromethane baths (at least 5) and successive drying in order to eliminate any potential contamination by contact with the product or the smoke. Then dried nails were finely pulverized in a bullet blender and weighed. Samples of 50 mg were analyzed by GC-MS. The nails concentrations of COC, BZE, EME, and AEME were 28.7, 7.3, 6.3, and 0.39 ng/mg for subject 1 and 34.5, 17.9, 2.5, and 0.24 ng/mg for subject 2, respectively. As it is the case for hair, cocaine was present at higher concentration when compared with the other analytes in nails. The precautions of treatment of the sample avoided the risk of external contamination, which is the main source of false interpretation in this analysis. This result shows that nail specimen is an alternative to hair to document chronic consumption of crack cocaine.

<table>
<thead>
<tr>
<th>Nails (ng/mg)</th>
<th>Urine (ng/ml)</th>
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<tbody>
<tr>
<td>COC</td>
<td>BE</td>
</tr>
<tr>
<td>Subject 1</td>
<td>28.7</td>
</tr>
<tr>
<td>Subject 2</td>
<td>34.5</td>
</tr>
</tbody>
</table>

Table II. Concentration of COC and Its Metabolites BZE, EME, CE, and AEME in Nails and Urine of the Two Studied Subjects

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References


